VOLGOGRAD STATE MEDICAL UNIVERSITY

DEPARTMENT OF PHARMACOLOGY AND BIOINFORMATICS

Methodological recommendations for students for practical classes
«Immunobiological and gene therapy drugs»

Thematic block: **Immunobiological drugs**

Class topic:

Immunobiological drugs. Serums, toxoids and immunoglobulins, chemical vaccines (antigens), bacteriophages, interferons, probiotics. Advantages and disadvantages of these groups of immunobiological drugs.

Pharmaceutical faculty

1. Class aims

- to teach students to analyze the action of immunobiological drugs (serums, toxoids and immunoglobulins, chemical vaccines (antigens), bacteriophages, interferons, probiotics) based on their pharmacological properties, mechanisms and localization of action;
- to teach students the general principles of the immunological basis of vaccination;
- to teach students to evaluate the effectiveness of the use of serums, toxoids and immunoglobulins, chemical vaccines (antigens), bacteriophages, interferons, probiotics. The advantages and disadvantages of these groups of immunobiological drugs, depending on their type and method of application;
- to teach students to evaluate the advantages and disadvantages of this group of immunobiological drugs;
- to become familiar with the need to conduct educational work with the population on issues of vaccination - as a significant factor in the fight against infectious diseases

2. TASKS

- For serums, toxoids and immunoglobulins, bacteriophages, interferons, probiotics, study:
  - classification and composition;
  - advantages and disadvantages of these groups of immunobiological drugs;
  - main mechanisms of action and application in medicine.
  - Study the features of development and production of the studied group of drugs.
- Study the basic terms and definitions used in the process of creating vaccines.
- Study the general requirements for the production, transportation and storage of serums, anatoxins and immunoglobulins, bacteriophages, interferons, probiotics.

3. THE FOLLOWING PRACTICAL SKILLS AND ABILITIES ARE PRACTISED IN THE CLASS

- ability to classify drugs of the studied group based on the mechanism of action, methods of application;
- ability to analyze the possibilities of using serums, toxoids and immunoglobulins, bacteriophages, interferons, probiotics;
- ability to analyze the advantages and disadvantages of serums, toxoids and immunoglobulins, bacteriophages, interferons, probiotics.

4. Class timetable:

Venue: classroom of the Department of Pharmacology and Bioinformatics.
Time of event: part 1 –2 AH

Formed competencies УК-1.1.3, УК-1.2.1, УК-1.2.2, УК-1.2.3., УК-1.3.1, УК-1.3.2., УК-6.1.1., УК-6.2.1, УК-6.2.2, УК-6.3.1, УК-6.3.2, УК-6.3.3, УК-6.3.4, ОПК-1.1.1., ОПК-1.2.1, ОПК-1.2.2., ОПК-1.3.1, ОПК-6.1.1, ОПК-6.2.1, ОПК-6.3.1, ПК-7.1.1, ПК-7.2.1, ПК-7.3.1.

4.1 Technological map of the lesson

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

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 by the teacher, a statement of the purpose of the lesson and answers to students' questions.

The importance of the topic in the system of training and activities of a pharmacist:

- informing the population on vaccination issues in accordance with the National Vaccination Calendar, on the use of bacteriophages and probiotics for both preventive and therapeutic purposes;
- draw the attention of pharmacists to the prohibition of dispensing drugs by pharmacies (clause 5, 6 of the RF Government Resolution of 22.12.2011 No. 1081 "On licensing pharmaceutical activities")

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

4.3.3 Разбор теоретического материала

Plan for analyzing theoretical material

1 Serum

- *general characteristics of serums;*
- *features of the immune response when using serums.*

2 Anatoxins:

- They are completely neutralized bacterial exotoxins with high immunogenicity. Detoxification of toxins, carried out by chemical and/or physical methods, should ensure the preservation of their antigenic activity and guarantee the absence of reversion of toxic properties.
- Toxoids should be maximally purified from ballast impurities by methods that ensure the preservation of their antigenic properties. To increase immunogenicity, toxoids are adsorbed on adjuvants - usually on aluminum hydroxide. The amount of toxoid in a unit of volume or in a vaccination dose is expressed in units of mass or established International Units (flocculating units - Lf, binding units - EU, etc.). Specific (immunogenic) activity of toxoids in the composition of adsorbed vaccines is expressed in IU (international units).

3 Immunoglobulins.

– Immunoglobulin G (IgG) is the most populous type of antibody in the circulatory system. It is a monomer and makes up about 75% of all immunoglobulins in the human bloodstream.

These antibodies provide long-term immunity due to their ability to remain in the blood for several weeks and bind to bacteria, viruses, and other antigens to destroy them. Immunoglobulin A (IgA) is a dimeric antibody that is usually secreted in the blood and other secretions, such as saliva, milk, and gastrointestinal secretions. Antibodies help protect the body from infections in the nasopharynx, lungs, gastrointestinal tract, and other locations. Immunoglobulin M (IgM) is a large pentameric antibody that usually appears in the blood in response to the first introduction of an antigen. IgM has a high affinity for antigen and can effectively destroy bacteria and viruses.

- Immunoglobulin D (IgD) is a monomeric antibody that is localized on the surface of B lymphocytes and plays an important role in stimulating immunity in response to an antigen.
- Immunoglobulin E (IgE) is a representative of monomeric immunoglobulins that plays a key role in allergic reactions. IgE binds to allergens such as pollen or food products and promotes the release of histamine and other chemical substances that cause allergy symptoms: itching, redness, and swelling.

4 Synthetic vaccines. Antigen molecules have low immunogenicity due to the relatively low molecular weight of antigens. In this regard, there are searches for increasing the immunogenicity of molecular antigens by artificially enlarging their molecules due to chemical or physicochemical bonding ("cross-linking") of the antigen with polymeric large-molecular carriers harmless to the body (such as polyvinylpyrrolidone), which would play the role of an assistant.

5 General requirements for the production of vaccines and toxoids

- All stages of vaccine and toxoid production must be validated to confirm the established requirements that guarantee their quality and safety of use. The conditions of the production process must meet the requirements set out in the OFS "Immunobiological medicinal products".
- Vaccines and toxoids are produced using working seed lots of microorganisms that must have the same characteristics as the strain from which the initial seed lot was obtained. Microorganism strains used as the initial seed lot must be identified and characterized, including information on their origin.
- Cultivation methods must ensure the preservation of the immunogenic properties of vaccine strains, the safety of the drug, and prevent contamination with foreign viruses, bacteria, fungi and mycoplasmas.
- Nutrient media for culturing vaccine strains must not contain ingredients that cause toxic, allergic or other adverse reactions in humans. If it is necessary to use such ingredients, it should be demonstrated that their amount in the final product is below the level that guarantees safety for humans.
- Animals used in production and testing are obtained from farms free from bacterial, viral, prion and other diseases dangerous to humans.
- When testing on laboratory animals, the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes should be taken into account: tests should be carried out in such a way as to use the minimum number of animals and cause them the least pain, suffering and harm.

6 Cell cultures

- Cell cultures used to obtain vaccines must be certified, deposited in state collections and approved for use for the above purposes by the authorized body and meet the requirements set out in the OFS "Requirements for cell cultures - substrates for the production of biological medicinal products".
- When culturing cells, it is not allowed to use native human blood serum, as well as penicillin and streptomycin. If it is necessary to use other antibiotics, they should be used in the minimum effective concentration. The presence of a pH indicator, such as phenol red, is allowed in culture media.
- Chicken or quail embryos used for production are obtained only from healthy birds from poultry farms that are free of infectious diseases in birds; the quality of the supplied embryos is confirmed by documents from the veterinary service on the sanitary condition of the livestock. When incubating embryos used for virus propagation, at least 2% of uninfected embryos are incubated in parallel with infected embryos as a control for the absence of contaminating viruses.

- When working with cultures of pathogenic microbes and toxins of biological nature, current sanitary and epidemiological rules must be observed.

7 Adsorbents

- Vaccines and toxoids may be adsorbed on aluminum hydroxide, aluminum phosphate, calcium phosphate or other suitable adsorbents, the safety and efficacy of which have been established for the appropriate route of administration.
- In the production of adsorbed vaccines and toxoids, a validated method of antigen adsorption is used, ensuring the regulated completeness of sorption throughout the shelf life of the ILP. The amount and sorption capacity of the adsorbent must ensure the maximum possible sorption of the antigen and its stability.
- The completeness of sorption is determined by indicating the antigen (antigens) in the supernatant of vaccines and toxoids, using biological, immunological and immunochemical methods.

8 Preservatives

- Antimicrobial preservatives are recommended for use in the production of adsorbed drugs and drugs produced in multi-dose packaging. It is not recommended to use preservatives in the production of lyophilized drugs and drugs produced in single-dose packaging.
- Indicators that significantly affect the quality of the final product, but cannot be identified, must be determined at intermediate stages of production, as indicated in the regulatory documentation.
- Medicines are produced in various dosage forms: lyophilizates, powders, solutions, suspensions, tablets, granules, spray. The lyophilizate can be released complete with a solvent approved for medical use, in the appropriate dosage for a given method of use. The solvent should not affect the quality of the drug.

9 Testing of vaccines taking into account the requirements for various dosage forms in accordance with the OFS.

- ***pH.*** The determination is carried out by the potentiometric method in accordance with the State Pharmacopoeia Monograph "Ionometry". Tests of liquid dosage forms are carried out with the undiluted drug; lyophilized forms - with the drug dissolved in the supplied solvent, and in its absence - in the solvent specified in the instructions for use.
- ***Loss of weight on drying*** - no more than 3.0% for lyophilized vaccines, unless otherwise specified in the regulatory documentation. For tablet dosage forms - no more than 4.0%, provided that all basic properties are stably preserved throughout the shelf life. The regulatory documentation specifies the determination method for all dosage forms.
- **Chemical indicators**. If necessary, indicate the requirements for the quantitative content of protein, nucleic acids, polysaccharides, etc. and describe the method for their quantitative determination (if they are not subject to inclusion in the section “Specific activity”).
- **Sterility.** Inactivated vaccines and toxoids for injection must be sterile! The test is carried out in accordance with the OFS "Sterility". If it is necessary to carry out control for the absence of mycoplasmas, this subsection is placed after the description of the control for sterility, without highlighting it in the heading.
- **Absence of foreign bacteria and fungi.** For live bacterial vaccines, the test is carried out in two stages. At the first stage, the test sample is sown on a thioglycolate medium, the tests are carried out according to the OFS "Sterility", keeping the crops at the appropriate temperature until the end of incubation. At the second stage, the grown bacteria are identified. The regulatory documentation specifies the requirements and methods of determination, as well as the procedure for recording and interpreting the results.
- **Microbiological purity.** For non-injectable dosage forms, the maximum permissible contamination of the drug and a list of types of microorganisms whose presence is unacceptable are indicated. The determination is carried out in accordance with the OFS "Microbiological purity".
- **Pyrogenicity or bacterial endotoxins.** The determination is carried out in accordance with the OFS "Pyrogenicity" or the OFS "Bacterial endotoxins". For drugs administered parenterally that do not contain endotoxins, the determination method, sample preparation and test dose of the drug,

permissible determination conditions (the value of permissible indicators of animal body temperature or the content of bacterial endotoxins in the corresponding units) are indicated.

- **Safety.** Specify regulatory requirements and describe in vivo and/or in vitro methods that allow assessment of the completeness of inactivation (for inactivated vaccines), acceptable residual virulence of microorganisms (for live vaccines), or the absence of exotoxins (for toxoids).
- **Toxicity.** Provide regulatory requirements and describe methods to prove the absence of toxic substances in the drug, indicating the animal species, test dose, route of administration, observation time, and criteria for acceptability of results. The determination is carried out in accordance with the Pharmacopoeia Monograph "Abnormal Toxicity".
- **Specific activity.** The requirements for specific activity and methods of its quantitative assessment in vivo and/or in vitro (e.g. quantitative antigen content, quantitative content of live microorganisms in a unit volume or vaccination dose, antigenic activity, immunogenic properties, etc.) are specified. The choice of methods used is determined by the type of preparation. In animal studies, their species, breed/line, quantity, body weight, age, and gender are specified. Sample preparation, doses, regimens, and methods of administration of the test preparations and standard samples (if used), methods of evaluation of results and calculations, and requirements for test results are described. When using test strains of microorganisms, their name and collection name, catalog number, test dose, and route of administration are provided. If the use of avian embryos is envisaged, the requirements for their age are provided; when using cell cultures, their name is provided.
- **Completeness of sorption.** The content of non-adsorbed antigens in the supernatant of adsorbed vaccines should not exceed 1%, unless otherwise specified in the regulatory documentation. A description of the method for determining the content of non-adsorbed antigens in the supernatant of adsorbed vaccines is provided.
- **Production strains of microorganisms and strains for control. Preservatives.** When using preservatives, their concentration should not be lower than the minimum effective and should not exceed the value indicated on the drug packaging by more than 15%. When using the antimicrobial preservative - thiomersal, the determination is carried out in accordance with the OFS "Quantitative determination of thiomersal in immunobiological medicinal products".

  Free formaldehyde - no more than 0.2 g/l (in preparations for children no more than 0.1 g/l). The determination is carried out in accordance with the OFS "Quantitative Determination of Formaldehyde in Immunobiological Medicinal Products".

  Phenol - no more than 2.5 g/l, if phenol was used in the preparation of vaccines and toxoids. The determination is carried out in accordance with the OFS "Quantitative Determination of Phenol by Spectrophotometric Method in Immunobiological Medicinal Products". In preparations containing diphtheria and tetanus toxoids, as well as pertussis suspension, the presence of phenol is not allowed.

  Aluminum - no more than 1.25 mg of aluminum (III) per dose, if an adsorbent containing aluminum was used in the preparation, and unless otherwise specified in the regulatory documentation. The determination is carried out in accordance with the OFS "Determination of Aluminum Ions in Adsorbed Biological Medicinal Products". Calcium - no more than 1.3 mg calcium (II) per dose, when using an adsorbent containing calcium, unless otherwise indicated in the regulatory documentation.
- **Solvents.** Solvents for lyophilized preparations are those approved for medical use with the appropriate route of administration. The type of solvent and requirements for its quality are indicated.
- **Package.** In accordance with the Federal Pharmacopoeia of the Russian Federation "Immunobiological medicinal products". It is not recommended to use ampoules for the release of the drug in multi-dose packaging.
- **Transportation.** In accordance with the OFS "Immunobiological medicinal products". The section specifies the document regulating the conditions and temperature of transportation; if necessary, indicate the duration of transportation at a temperature different from that specified in the reg-

ulatory documentation. Liquid adsorbed vaccines and toxoids must be transported under conditions that exclude freezing.

- **Storage.** In accordance with the OFS "Immunobiological medicinal products". The section specifies the regulated storage conditions, primarily the storage temperature that ensures the preservation of the drug's activity during the declared shelf life. Unless otherwise specified, the storage temperature should be between 2 and 8 °C. Liquid vaccines (as a rule) and toxoids should be stored under conditions that exclude freezing.

10 Bacteriophages.

Modern antimicrobial drugs of natural origin. These are microorganisms capable of precisely destroying pathogenic bacteria. Bacteriophages are used in the prevention and antibacterial therapy of diseases caused by pathogenic bacteria.

– **Structure of a bacteriophage**

Bacteriophages have a cubic, thread-like or tadpole shape. The head of the bacteriophage contains nucleic acid (DNA or RNA) enclosed in a protein shell. Below is the tail process, consisting of an internal rod and a contractile sheath. The bacteriophage moves with the help of fibril legs, fastened in the center by a basal plate. The size of the bacteriophage is hundreds and thousands of times smaller than microbial cells.

– **How do bacteriophages work?**

Bacteriophages (from Latin phagos - devouring bacteria) - special viruses that can reproduce only in the presence of a certain type of pathogenic bacteria. Bacteriophages reproduce their own kind only at the expense of bacteria.

6 stages of bacteriophage work

- ✓ *Attachment of bacteriophages to bacterial cells*
- ✓ *Injection of bacteriophage nucleic acid into bacteria*
- ✓ *Synthesis of protein and nucleic acid components of bacteriophages*
- ✓ *Combination of protein and nucleic acid components of phages*
- ✓ *Assembly of new phage particles*
- ✓ *Release of mature phages and death of bacteria*

Bacteriophages are used for the treatment and prevention of infectious diseases in the following areas: gastroenterology (cholecystitis, gastroenterocolitis, intestinal dysbacteriosis), urogynecology (cystitis, pyelonephritis, colpitis, urethritis, endometritis), surgery (abscess, panaritium, paraproctitis, osteomyelitis, mastitis, peritonitis, furuncles, burns, purulent wounds, prevention of nosocomial infections), otolaryngology (sinusitis, tonsillitis, pharyngitis, laryngitis, bronchitis, sore throat, sinusitis, otitis), pulmonology (tracheitis, pleurisy, laryngitis, bronchitis, pneumonia), infectious diseases of the gastrointestinal tract (bacterial dysentery, dysbacteriosis, enterocolitis, colitis, dyspepsia, purulent-inflammatory diseases of the skin and mucous membranes). pyoderma, conjunctivitis, keratoconjunctivitis, sepsis).

IN NATURE	IN MEDICINE
Are present everywhere in our world - in the ocean, soil, deep-sea sources, drinking water and food. The most common form of life on Earth; the biosphere contains from 1030 to 1032 phage particles. The oldest known microorganisms - their age is estimated at about 3 billion years. Play a key role in maintaining the balance of all ecosystems studied by man. Control the amount of microbial flora and restrain its pathological growth	The history of using bacteriophages goes back more than 100 years. The global medical and scientific community is actively researching phages as antimicrobial agents. Biotechnologists have learned to create medical antibacterial drugs based on bacteriophages and use them to treat patients. Modern medicine allows the use of bacteriophage therapy against most bacterial infections.

Facts about bacteriophages	
Bacteriophages – antibacterial agents and natural antiseptics Bacteriophages are compatible with all medications. The use of bacteriophages does not limit the use of other medications and does not affect their effectiveness It affects only pathogenic bacteria that are sensitive to them, causing infectious diseases, destroying them from the inside	Safe and non-toxic, side effects are rare, used in newborns, pregnant and lactating women The action of bacteriophages does not affect the beneficial microflora of the body, unlike antibiotics Bacteriophages are eliminated from the body naturally

11 Probiotics.

– *Pharmacological properties of probiotics*

Probiotics contain a culture of live non-pathogenic bacteria, representatives of the normal microflora of the human intestine, and are intended to correct, i.e. normalize, the qualitative and quantitative composition of the human microflora in case of their violation, i.e. in dysbacteriosis.

– *Use of probiotics*

Probiotics are used for both preventive and therapeutic purposes in dysbacteriosis of various etiologies. The most common probiotics include "Colibacterin", "Bifidumbacterin", "Lactobacterin", "Bificol", "Subtilin", which include E. coli, bifidobacteria, lactobacilli, spores, respectively. Currently, probiotics in the form of fermented milk products are widely used: "Bio-kefir", kefir "Bifidox".

– *Purpose and storage conditions of probiotic preparations*

Considering that probiotics contain live microbial cells; they must be stored in gentle conditions. Probiotics are prescribed orally in long courses (from 1 to 6 months) 2-3 times a day in combination with other treatment methods.

4.3.4 Independent work:

1. Conduct a search and write down the names of vaccines based on viral vectors and based on messenger RNA (mRNA)
2. Fill in the table of the National Immunization Calendar in the Russian Federation for diseases prevented by vaccines based on viral vectors and based on messenger RNA (mRNA). The information is entered into students' workbooks.
3. Working with advertising brochures of medicines on this topic.

4.3.5 Checking the completion of independent work.

4.3.6 Summing up the lesson. Answers to questions.

4.3.7 Concluding remarks by the teacher.

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