MINISTRY OF THE PUBLIC HEALTH OF UKRAINE
ZAPOROZHYE STATE MEDICAL UNIVERSITY

CHAIR OF MICROBIOLOGY, VIROLOGY AND IMMUNOLOGY

Module I

Collection of methodical recommendations

for practical classes

on microbiology, virology and immunology

for the students of 2nd year of the medical faculty

Part II

Zaporizhzhia

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The methodical manual for practical lessons on microbiology, virology, immunology for the medical students of II-III year of the study are approved by the Central Methods Board of ZSMU as a methodical manual on practical lessons for students of the medical faculty.

The independent practical work of students is an important part of the syllabus in the course of microbiology, virology and immunology. It helps students to study this fundamental subject.

The systematic independent work enables to reach the final goal in the students' education. It is also important while preparing the students for their future clinic work with patients.

These theoretical material, questions and tests help students to get ready for examination.

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
ЗАПОРІЗЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ

Кафедра мікробіології, вірусології та імунології

Модуль І

Збірник методичних рекомендацій для підготовки практичних занять з мікробіології, вірусології та імунології для студентів ІІ курсу міжнародного факультету, спеціальність «Лікувальна справа»

ІІ частина

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Затверджено на засіданні Центральної методичної Ради ЗДМУ (протокол № від 20 р.) та рекомендовано для використання в навчальному процесі.

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Збірник методичних рекомендацій для підготовки практичних занять з мікробіології, вірусології та імунології для англомовних студентів II-III курсів міжнародного факультету, спеціальність «Лікувальна справа». II частина. Модуль 1 / Єрьоміна А.К. [та ін.]. – Запоріжжя, 2016. – 94 с.

Plan

of the lectures in microbiology for foreign students of the medical faculty for spring semester

№	Theme	Amount of hours
1.	Subject and problems of microbiology in historical development. Structure and functions of the bacterial cells. Classification of microorganisms. Physiology of microorganisms. Nutrition, respiratory, growth and reproduction.	2
2.	Antibacterial chemotherapy of an infections diseases. Antibiotics. Genetics of bacteria. Genetic engineering. Biotechnology.	2
3.	The infection. The forms of infections. The infectious process. Pathogenicity and virulence of bacteria.	2
4.	Immunity. Immune system. Antibodies and antigens, their nature and properties. Immune diagnostics.	2
5.	Allergy. Reactions of immunity. Immunoprophylaxis and Immunotherapy of infectious diseases. Immunobiological preparations.	2
6.	History of virology. Morphology, structure, chemical composition, base of classification and reproduction of viruses. Antivirus immunity.	2
7.	Influensavirus and Parainfluenza viruses. Acute respiratory virus infections(respiratory-syncytial virus, reovirus, rhinovirus, adenovirus). Virus of measles. Mumps virus. Rubivirus.	2
8.	Herpesviruses. Virus of chickenpox. Smallpox.	2
9.	Virus of poliomyelitis, ECHO and Coxsackie. Hepatitis Viruses.	2
10.	Viruses of encephalitis, hemorrhagic fever. Viruses of immunodeficiency. AIDS. Principles and methods of the laboratory diagnostics. Oncogenic viruses.	2
	TOTAL	**20**

Plan

of practical classes in microbiology for foreign students of the medical faculty for spring semester

№	Theme	Amount of hours
1.	Microbiological laboratory equipments and instructions for work. Structure of biological light to the microscope and rules of work with him. ***Bacterioscopical method of research.*** Microscopy of the prepared given. Morphology of bacteria. Preparations for a microscopy. Method of staining by Gram.	2,5
2.	Structure of the bacterial cell. Complex methods of staining by Anjesko, Neisser, Burri-Gins and Ziehl-Nilsen. Morphology of spirochetes, riskettsia, fungi and the protozoa.	2,5
3.	***Bacteriological method of research.*** Nutrition of bacteria. Nutrient media and methods of bacteria cultivation. Methods of sterilization. Asepsis and antiseptic. Disinfection. Chemotherapy. Chemotherapeutic preparations.	2,5
4.	Growth and reproduction of bacteria. Methods of isolation and cultivation of pure cultures of aerobes. Respiration of bacteria. Methods of isolation and cultivation of anaerobes. Biochemical properties of microorganisms.	2,5
5.	Genetics of microorganisms. Methods of biotechnology and gene engineering. ***Genetic method of diagnostics.*** Polymerase chain reaction (PCR). Polymerase chain reaction with reverse transcriprase (RT-PCR). Polymerase chain reaction in the real time.	2,5
6.	Ecological microbiology. Microflora of an environment and the human body. Methods of sanitary bacteriological research. Antibiotics. Bacteriophages.	2,5
7.	**Submodule 1. Morphology and physiology of microorganisms.**	**2,5**
8.	Infections, infectious and epidemiological process. Pathogenic factors of microorganisms. Mechanisms of pathogenesis of an infectious diseases. Experimental infection of laboratory animals.	2,5
9.	Immunity. Kinds and forms of immunity. Types of immune answer. Innate immunity. Factors of non specific organism defence. Cells and receptors of innate immunity.	2,5
10.	Adaptive immunity.T- and B-lymphocytes. Description of antigens. Presentation of antigens. Activating of lymphocytes. Antiinfectious immunity.***Immunological method of research*** – serological reactions. Immunoglobulins. Serological reactions of agglutination and precipitation,	

	immune lysis and compliment fixation test, their description and practical use. Coomb's test. Immunohematology. Application of monoclonal antibodies in immunocytochemical and immunohistochemical reactions.	2.5
11.	Immune serum and immunoglobulins. Reaction of flocullation (neutralization). Hypersensitivity. Allergic reactions for immunodiagnosis of infectious diseases. Autoimmune phenomena. Principles of the use of antibodies as medical, preventive and diagnostic preparations.	2,5
12.	Vaccines. Principles of making and application of vaccines. Immunobiological preparations. Immune status of man. Estimation of immune status. Immunomodulators. Immunocorrection. Transplantation immunology.	2,5
13.	Virologic laboratory. Morphology and ultrastructure of viruses. Principles of classification. Virologic methods of research. Cultivation of viruses in culture cells and in chick embryos. Indication of viral reproduction.	2,5
14.	Antiviral immunity. Immunoreactions in virology: hemagglutination reaction, hemagglutination inhibition assay, hemadsorption phenomenon, neutralization test. Radioimmunoassay, direct and indirect immunofluorescence, enzyme immunoassay. Enzyme linked immunosorbent assay (ELISA), immunoelectroblot technigues. Immunochromatography analisis. Immunology of tumours.	2,5
15.	**Submodule 2. Infection, immunity and general virology.**	**2,5**
16.	Laboratory diagnostics of flu and parainfluenza. Laboratory diagnostics of adenoviruses Laboratory diagnostics of mumps, measles and rubella.	2,5
17.	Laboratory diagnostics of chikenpox, smallpox, herpes, zoster, poliomyelitis, Coxsakie and ECHO.	2,5
18.	Laboratory diagnostics of viral hepatitis A, B, C, D, E, F, G, rabies and arboviruses infections.	2,5
19.	Laboratory diagnostics of AIDS. Oncogenic viruses. Viral genetic theory of tumours origin.	2,5
20.	**Final module control I.**	2,5
	TOTAL	**50**

INDEPENDENT WORK
on microbiology for foreign students of II course of the medical faculty

№	Theme	Hours quantity
1.	Morphology of microorganisms. Simple and complex methods of coloring the bacteria.	6
2.	Structure of bacterial cell. Complex methods of coloring the bacteria.	4
3.	Morphology of spirochetes and rickets.	4
4.	Morphology of fungi and elementary.	2
5.	Nutrition of microorganisms, nutrient mediums and methods of cultivation bacteria. Devices and methods of sterilization.	4
6.	Action of some physical and chemical factors on microorganisms, disinfection.	2
7.	Antibiotics and Chemotherapy.	6
8.	Genetics of microorganisms. Methods of biotechnology and gene engineering.	4
9.	An infection. Experimental infection of laboratory animals.	4
10.	Immunity, its kinds and forms. Nonspecific factors of protection of an organism.	6
11.	Immune reactoins for infectious diseases.	2
12.	Vaccines. Principles of manufacturing and application of vaccines. Immunobiological preparations.	2
13.	Prion diseases of humans and animals.	2
14.	Modern methods of the laboratory diagnostics of infectious diseases	2
	Total	**50**

***Theme*: Infections, infectious and epidemiological process. Pathogenic factors of microorganisms. Experimental infection of laboratory animals.**

Questions of the learning.

1. Infections. Infections process, infectious disease, their forms. Propagation of microbes in organism.
2. Pathogenicity and virulence, factors of virulence.
3. Role of microorganisms, social and natural environments in development of infectious process.
4. Transmission of an infection. Classification of infectious diseases.
5. Mechanisms of pathogenesis of an infectious diseases. Dynamics and types of infectious diseases.
6. Purpose and methods of laboratory animal's inoculation.

Disease, or illness, is characterized by changes in the host that interfere with normal function. Microbial parasites are transmissible from one individual of the host species to another; the establishment of a pathogen in the body of a fresh host is called ***infection.***

An ***infectious disease*** is a disease that involves pathogen infection; any disease caused by a pathogen is an infectious disease; any disease not caused by a pathogen is a non-infectious disease. Infectious diseases are often notable in that the causative agent is not diluted with time, i.e., the disease process increases the prevalence of the causative agent (i.e., the pathogen replicates at the expense of the host). However, not all infectious diseases are spread from host to host; additionally, not all infectious diseases are associated with pathogen infection of the host (e.g., intoxication can result from exposure to secreted toxin rather than the secreting organism).

The distinguishing signs of infectious diseases are:

(a) presence of certain alive causative agent;

(b) infectious nature;

(c) cyclical duration (incubation, prodromal period, acme, convalescence);

(d) development of immune response.

The dynamics of development of infectious diseases.

1. Icubation period begins since the moment of causative agent invasion to human organism till the appearance of first signs of the disease. The duration of the period lasts from several hours to decades of years depending on nosology form.

2. Prodromal period is the period of appearance of first non specific symptoms of illness, but full-blown illness has not-yet begun. Causative agent colonize sensitive cells and does not extend to environment (exception - measles, viral hepatitis A). Antibodies to causative agent are not identified. Not all diseases have prodromal period. If host .efenses are successful, an infection may disappear without progressing beyond this vague feeling of being sick.

3. Acme is period of appearance and the peak of disease specific symptoms. Microbes divide intensively and extend to environment. Specific immune globulin M is small titer appear in blood. In the end of the period the immune globulin M changes to immune globulin A and G.The occurrence of an acme results either from the infection being self-limiting, or the host immune system or medical procedures bringing the infection under control.

4. Convalescence is the period of recovery (the time during which the host repairs the damage wrought by the infection). The microbes stop their division and die. The microbial allocation stops or the microbial carriage is formed. The titer of immune globulin G and A increases. At many diseases the delayed hypersensivity reaction appears. Individuals are not necessarily, depending on disease/pathogen, no-longer contagious.

On the mechanism of causative agent transmission

all infectious diseases are divided to:

1. Intestinal infections. Mechanism of transmission is fecal-oral, ways of transmission - through water, foodstuff, contacts in household.

2. Airborne (droplet) infections (infections of respiratory tract). Mechanism of transmission is aerial, ways of transmission - through airborne droplets and dust.

3. Blood infections. Mechanism of transmission is hemic (through the bites of blood-suckling insects).

4. Infections of external covers. Mechanism of transmission is percutaneous (through skin), ways of transmission - sexual, parenteral, contact (during salivation at rabies).

Infectious dose of causative agent plays important role in development of infectious process. *Infectious dose* is the minimal amount of microbial cells that are able to cause infectious process. Infectious dose depends on microbial species.

On origination and type of spreading all infections are divided into:

(a) *exogenous* infection when the individual is infected by normal micro-flora or by L-forms of bacteria;

(b) *autoinfection* - is the kind of exogenous infections which appears as a result of autoinfecting through trans-location of causative agent from one microbiota to another (for example, from oral cavity to wound surface).

On localization of causative agent in host organism all infections are divided into:

(a) local infection - it is confined to a certain area (e.g., a pimple),

(b) systemic infection - during which microbes are spread throughout the body in the blood or lymph.

Systemic infection is divided into:

1) ***bacteremia*** **(**it is the presence, without multiplication, of bacteria in the blood) or *viremia* (the presence of virus in the blood (this would be in the acellular portion of the

blood; viruses require cells to multiply and that these cells are not necessarily blood cells);

2) ***sepsis*** - generalized acute or chronic infectious disease during which bacteria multiply in host blood. **Sepsis** is divided into septicemia, septicopyemia and toxemia.

Septicemia (primary sepsis) is the growth of bacteria in the blood (blood poisoning).

Septicopyemia (secondary, metastatic sepsis) occurs as a result of local infectious process generalization, secondary purulent foci appear in internal organs.

Toxemia (or toxic shock) is the presence of bacteria and their toxins in the blood that causes the fall of arterial pressure.

On number of causative agents species all infections are divided into:

a) *mono-infection*, when infectious process is caused by one mi-crobial species,

b) *mixed infection*, when infectious process is caused by a combination of two or more microbial species.

On repeated appearance of the disease, caused by the same agents,

all infections are divided into:

a) primary infection - an infection of a not-currently infected person;

b) *secondary infection* - an infection (with new causative agent) that quickly follows a primary infection;

c) *reinfection* - the repeated infection by the same causative agent after the recovery from the disease (dysentery, gonorrhea);

d) *superinfection* - a secondary infection caused by the treatment of a primary infection (e.g., as in the superinfection by an antibiotic-resistant organism following antibiotic treatment);

e) *recidivation* - return of the disease without secondary infection with the microbes, remained in organism;

f) *coinfection* -is the simultaneous invasion to the host organism of two or more infectious agents.

On duration of host-microbial interaction all infections are divided into:

a) *acute infection*, that develops rapidly but is soon over (for example, food poisoning);

b) *chronic infection* that develops slowly and is not soon over (mycoses, tuberculosis);

c) *microbial (bacterial, viral) carriage* - the state when the organism allocates the causative agent after clinical recovery.

On display of the illness all infections are divided into:

a) *manifest infection* that has expressed signs and symptoms;

b) *inapparent (sub-clinical) infection* that does not display signs or symptoms or, at least, all of the signs typically associated with a given syndrome.

On source of infection all infections are divided into:

a) *human* (anthroponoses);

b) *animal* (zoonoses);

c) *environmental* (sapronoses).

Causative Agents of diseases and their properties.

Causative agents of infections and infectious processes are (a) pathogenic microorganisms with gaining access to the host, adhering to and colonizing cell surfaces, invading tissues, and producing toxins and other harmful metabolic products, that can initiate infectious disease; and (b) conditionally pathogenic microbes. The main properties of causative agents are:

1) pathogenicity;

2) virulence;

3) toxicity.

The term ***pathogenicity*** denotes the ability of a parasite to cause disease. Pathogenicity is a taxonomically significant attribute, being the property of a species; thus, the bacterial species *Corynembacterium diphtheriae* is said to be pathogenic for man. Pathogenicity is higher in some environments (e.g., infection of the blood) than others (e.g., presence in the lumen of the gastrointestinal tract), or pathogenicity is higher on some hosts but not on others depending on host susceptibility (in general or specifically towards a particular pathogen).

Pathogenicity can also depend on the number of organisms present, where many organisms have a greater potential of bypassing host defenses than fewer organisms of the same kind. A commensal, more or less by definition at a given place and given time, has a pathogenicity of effectively zero. For an organism to be pathogenic it must be able to invade a host, multiply in the host, evade host defenses, and harm the host in some way.

The individual strains of a bacterial species may, however, vary widely in their ability to harm the host species, and this relative pathogenicity is termed virulence. ***Virulence*** is accordingly an attribute of a strain, not a species; one may speak of a highly virulent, a weakly virulent, or even an avirulent strain of *Corynebacterium diphtheriae.*

Microbial virulence is the relative intrinsic ability of a microorganism to cause disease (gaining access to the host, adhering to and colonizing cell surfaces, invading tissues, and producing toxins and other harmful metabolic products). Organisms of high virulence have evolves efficient mechanisms for circumventing normal host defenses. Virulent organisms are adept at gaining entry and doing damage even when the inoculum is small.

The terms pathogenicity and virulence are closely related with pathogenicity referring to an organism's binary ability to cause disease (or not) given specific circumstances and virulence referring to the degree of disease caused (also dependent on specific circumstances).

In general, the virulence of a strain of a pathogenic species is determined by 2 factors: its **invasiveness,** or ability to proliferate in the body of the host, and its ***toxigenicity,*** or ability to produce chemical substances - *toxins* - that damage the tissues of the host. It is characteristic of bacterial toxins that they are capable of damaging or killing normal host cells (i.e., the cells of a host that has not previously been exposed to the infectious agent in question).

Certain pathogenic microorganisms, however, cause damage to the vertebrate host by a mechanism that is more indirect and does not come into play unless or until the host has previously experienced specific infection.

This mechanism is known as *hyper-sensitivity*, or *allergy*, and involves an immune response by the already sensitive host to a cell component of the parasite which is *nontoxic* for a normal host.

The units of virulence measurement are:

1) ***DLM (minimal lethal** dose)* - the smallest amount of microbial cells that in certain way of infestation causes the death of 95 % susceptible animals during the termed time;

2) ***LD50 (lethal** dose **50** %)* - the number of pathogens required to cause lethal disease in half of the exposed animals;

3) ***DCL (certain lethal dose)*** - the number of pathogens required to cause death in 100 % of the exposed animals.

Virulence factors may distinguish a pathogenic microorganism from otherwise identical non-pathogenic microorganisms by allowing pathogens to invade, adhere to, and colonize a host, and then harm the host. Pathogens may harm the host by direct or indirect means.

Virulence factors include:

1. ***Pili*** and ***capsules*** (used in adhesion to hosts - glycocalyx and fimbriae, i.e., attachment pili and to prevent phagocytosis).

2. ***Adhesins*** - the substances that help the bacterial adhesion on epithelial cells and their division otheir surface (colonization). There are two adherence mechanisms: (a) non-specific (by chemical or physical means) and (b) specific (by means of adhesins). Typical mechanisms of adherence are specific such that just as viruses may adhere to some cells but not others, bacteria may adhere to some cells (or tissues) but not to others. Following contact with a host a crucial first step in an infection is adherence to the host. Failure to adhere to the host typically results in an inability to causes disease as well as removal from the host. Typically, the host employs mechanisms designed to thwart adhesion.

3.Enzymes of pathogenic microbes promote invasion. Such enzymes are:

a) hyaluronidase (it specifically splits hyaluronic acid that is the component of intercellular substance and by this way increases the permeability of mucous membranes and connecting tissue;
b) neuraminidase (by means of this enzyme the microorganisms can invade cells and intercellular space.

The 2nd group of enzymes is created by latter that cause formation of toxic (metabolic) products:

a) urease (hydrolyzes urea);

b) amino acids dexarboxylase (promotes accumulation of toxic biogen amines).

4. ***Aggressins*** - are virulence factors that suppress host defense. This are substances of different chemical structure that are the part of superficial structures of bacterial wall: capsule and cell wall. They suppress migration of leukocytes and phagocytosis.

Enzymes, produced by microbes, also refer to aggressins:

a) proteases (destroy antibodies, complement);

b) coagulase (coagulates blood plasma);

c) fibrinolysin (dissolves fibrin conglomerates);

d) lecetinase (acts on lecetin).

5. ***Toxins*** are substances produced, for example, by microorganisms, that are poisonous to host organisms. About 220 bacterial toxins are known. About 40 % disrupt plasma membranes. **Bacterial toxins may be classified as either exotoxins or endotoxins.**

The names "exotoxin" and "endotoxin" to designate these two classes of toxic substances can be misleading, since there is now good evidence to show that many "exotoxins" are associated with the bacterial cells during growth and are liberated only after death and lysis of the bacteria.

Exotoxins can, however, be distinguished from endotoxins by their chemical nature. The former are simple proteins, whereas the latter are molecular complexes which contain protein, lipid, and polysaccharide. Nevertheless, these names are now so firmly entrenched that they are not likely to be abandoned.

Exotoxins are soluble substances - proteins, often enzymes (for example, hemolysins that catalyze the lysis of erythrocytes), produced inside of cells and which do their damage (as with endotoxins) only upon release from the cell. Exotoxins range in

toxicity up to and including extremely lethal. They are produced predominantly (though not exclusively) by gram-positive bacteria; exotoxins act by a variety of mechanisms and symptoms that result from exposure to an exotoxin depend on the structure of the exotoxin one is exposed to.

Many exotoxins are antigenic, i.e., antibodies can be made to them. However, typically exotoxins are produced in such small quantities that the host fails to develop an immune response against them. A way around this is to vaccinate using relatively large quantities of inactivated toxins, i.e., no-longer-toxic toxins, that are still antigenically intact. Such inactivated toxins are termed *toxoids.* Toxoids are typically produced by exposure to chemicals such as formaldehyde. Vaccines that employ toxoids include the tetanus and diphtheria vaccines.

Exotoxins can be distinguished in terms of the specific tissues they act against:

Cytotoxins are the exotoxins that disrupt host cells (they block the protein synthesis on cellular level). They are divides to: (a) antielongators that block the enzyme tranferrase, responsible for elongation of peptide chain on ribosome (diphtherin): (b) enterotoxins that act on tissues of the gastrointestinal tract, various food poisonings and diarrhea are caused by enterotoxins *{Staphylococcus aureus);* (c) dermatonecrotoxins *(Streptococcus pyogems)* that affect the skin.

Membranotoxins increase the permeability of superficial cell membranes with subsequent destruction. They are divided into: (a) hemolysins (increase permeability of erythrocytes); (b) leukocidines (increase permeability of leukocytes).

Toxins - functional blockers are divided into:

(a) thennoresistant enterotoxins and thermolabile enterotoxins that activate cell adenylatcy-clase, increase the level of cAMP, increase the permeability and diarrhea occurs;

(b) toxic blockers that activate cell adenylatcyclase;

(c) neuro-toxins that act on nervous system tissue. Examples of neurotoxins are the botulism and tetanus toxin, preventing muscle contraction and muscle relaxation, respectively.

Toxins — exfoliatines and erythrogenines - are produced by *Staphylococcus aureus* and *Streptococcus pyogenes.* They influence on cell-to-cell and cell-to-intercellular substances interaction.

Endotoxins are the lipid A portions of lipopolysaccharide; endotoxins are associated with Gram-negative bacteria; endotoxins arc weak except in large doses and produce similar effects independent on the producing organism; large doses are especially a problem given gram-negative septicemia. The endotoxins are relatively nonspecific, all producing much the same clinical and pathological symptoms when injected into experimental animals.

Endotoxins cause damage that is a consequence of the body using endotoxins as a signal for gram-negative bacterial infections; excessive amounts of endotoxin cause the body to overreact and damage itself. Endotoxins typically are released following bacterial division or lysis. Antibiotics that lyse gram-negative bacteria can produce a toxemia that increases symptoms rather than alleviating them.

Symptoms can include severely reduced blood pressure (endotoxic shock), fever, tissue damage, and death. Gram-negative septicemia is an acute, difficult to treat killer. There is no such thing as an endotoxin toxoid nor a vaccine against endotoxin.

Role of Macroorganism, social and natural environments

in development of infectious process.

Microbial invasion to human organism not always leads to the development of infectious disease. Non-susceptibility of human organism to causative agents is determined by immune system status. Many factors (both internal and external) influence on human immune system.

To ***internal*** *(endogenous)* ***factors*** relate:

a) gender (during periods, pregnancy or delivery human organism becomes more susceptible to diseases caused by staphylococci and streptococci);

b) age (children are more susceptible to dysentery, pneumonia, diseases caused by staphylococci and streptococci; it is associated with functional inferiority of child immune system;

c) HLA-phenotype;

d) state of non-specific antiinfectious defense system;

e) internal pathology;

f) endocrine system state (diabetes mellitus, thyroprivia), stress-reactions.

To ***external*** *(exogenous) factors* relate:

a) overheating;

b) over-cooling;

c) hypovitaminoses;

d) protein starvation;

e) alcoholism;

f) radiation;

g) industrial harm agents;

h) chronic intoxication.

Social factors that influence on spreading of infectious diseases are:

a) social disturbances (nervous stress, poverty);

b) state of vaccination (for example, against tuberculosis);

c) sanitary and hygienic measures (canalization, water supply).

***Practical lesson* # 8**

***Theme*: Infections, infectious and epidemiological process. Pathogenic factors of microorganisms. Experimental infection of laboratory animals.**

Questions of the learning.

1. Infections. Infections process, infectious disease, their forms. Propagation of microbes in organism.
2. Pathogenicity and virulence, factors of virulence.
3. Role of microorganisms, social and natural environments in development of infectious process.
4. Transmission of an infection. Classification of infectious diseases.
5. Mechanisms of pathogenesis of an infectious diseases. Dynamics and types of infectious diseases.
6. Purpose and methods of laboratory animal's inoculation.

Independent work.

1. Name the term:

a) Infection - _____
_____

b) Infectious process -
_____
_____

c) Infectious disease -
_____
_____
_____

2. Name the factors of the development of infectious process.

#_____
_____

#_____

#_____

3. Name the components of epidemiological process.

#_____
_____
#_____
_____
#_____
_____

4. What is the pathogenicity? **5. What is the virulence?**

_____

_____

_____

_____

_____

_____

_____

6. Name factors of pathogenicity:

_____

_____

_____

6a. Fill in the table. **Principal properties of pathogenic microorganisms.**

7. What is the toxin?

_____

_____

8. Fill in the table.

Characteristic of bacterial toxins

	Exotoxins	Endotoxins
Producents		
Localization		
Chemical nature		
Stabilization at 100º C		
Neutralization by antibodies		
Toxicity		

9. Name the stages of development of infectious diseases:

_____
_____
_____
_____

10. Transmission of an infection._____

_____
_____

Theme: **Immunity. Kinds and forms of immunity. Types of immune answer. Innate immunity. Nonspecific factors of organism defense against microbial agents. Cells and receptors of innate immunity.**

Questions of the learning.

1. Immunity. Central and peripheral organs of immune system.
2. Kinds and forms of immunity.
3. Types of immune answer. Innate immunity.
4. Nonspecific factors of organism defense against microbial agents. Phagocytosis and tissues of the immune response, their functions.
5. Cells and receptors of innate immunity.

The term ***immunity*** (L.*immunis* freed from) usually means resistance of the body to pathogenic microbes, their toxins or to other kinds of foreign substances.

Insusceptibility to infectious diseases depends on many factors grouped under the names of **resistance** and **immunity.**

Resistance is the insusceptibility of the body to the effect of pathogenic factors. Resistance is associated with the anatomical-physiological characteristics of the body, development of the central nervous system, and endocrine glands. It depends on the phylogenetic development of the animal, the individual and functional state of the body, and in man it depends also on social factors.

Mental traumas predispose to somatic and infectious diseases; chronic hunger and vitamin deficiencies lead to a decline in resistance; intoxication by alcohol, opium, cocaine and other narcotics has a negative effect on human resistance.

Types and Forms of Immunity

Modem classification divides immunity into two types according to origin: **(1)** ***species inherited (innate)*** **and (2)** *acquired.*

Species immunity is insusceptibility of certain species of animals to diseases which attack other species. It is transmitted by heredity from one generation to the next.

An example of species immunity is insusceptibility of man to cattle plague, chicken cholera and infectious horse anaemia.

On the other hand, animals are not infected by many human infections such as enteric fever, scarlet fever, syphilis, measles, etc.

The underlying factors of the mechanisms of species immunity (hereditary resistance) to infectious diseases are the absence in the organism's cells of receptors and substrates necessary for the adsorption (attachment) and reproduction of the causative agent, the presence of substances which block the reproduction of pathogenic agents, and the ability of the macroorganism to synthesize various inhibitors in response to the penetration of the pathogenic microbes.

Acquired immunity is subdivided into **natural and artificial.**

Natural immunity is divided into

(1) **active,** that is acquired following an obvious infection or latent disease without clinical manifestations,

(2**) passive immunity** of the newborn (maternal, placental), i e. immunity acquired from the mother in the period of intrauterine development.

The duration of this type of immunity is short. After about six months this immune state disappears and children become susceptible to many infections (measles, diphtheria, scarlet fever, etc.).

COMPARISON OF ACTIVE AND PASSIVE IMMUNITY

Active immunity	Passive immunity
Produced actively by the host's immune system	Received passively by the host. No participation by the host's immune system the host's immune system
Induced by infection or by contact with immunogens (vaccines,allergens)	Conferred by introduction of readymade antibodies
Affords durable and effective protection	Protection transient and less effective
Immunity effective only after a lag period (time required for generation of antibodies)	Immunity effective immediately
Immunological memory present; Subsequent challenge more effective (booster effect)	No immunological memory; subsequent administration of antibody less effective due to 'immune elimination'
Negative phase' may occur	No negative phase
Not applicable in immunodeficient hosts	Applicable in immunodeficient hosts

Artificial immunity is reproduced by active or passive immunization.

Immunity is manifested on the cell, molecular, and organism levels. The immune system is a sum total of lymphoid organs consisting of central (the thymus, bone marrow) and peripheral (lymph nodes, spleen, lymphocytes of peripheral blood) organs.

The systems of T-lymphocytes determined cell immunity in tuberculosis, leprosy, brucellosis, tularaemia and other diseases. The Fabricius' pouch in birds (its analogue in mammals are the Peyer's patches) is the central organ of humoral immunity.

The system of B-lymphocytes is responsible for humoral immunity against most bacterial infections and intoxications.

Depending on which agents the defense forces of the macroorganism are directed against immunity is subdivided into ***antibacterial, antitoxic, antiviral*** and ***antiparasitic immunities.***

This division of immunity by no means excludes the unity of all defense reactions and their interrelations. Absolutely autonomous forms of immunity do not exist, but all of them are interrelated and manifest their protective action in the entire organism with participating of all systems.

During some infectious diseases antibacterial immunity occurs to be most expressed, while in others - antitoxic immunity occurs. This differential manifestation of immunity is determined by biological peculiarities of the causative agent and by defense reactions which were formed during evolution of microbe and host.

Antibacterial immunity. During an active manifestation of host defensive forces a typical clinical picture of the disease does not ensue, and infection does not lead to disease in the human. In the blood and the reticuloendothelial system microbes are exposed to action of cellular and humoral factors.

Sterile immunity *is* that type of immunity in which the host has completely freed itself of the causative agent. Immunity after measles, pertussis, cholera, smallpox, etc., is an example of this kind of immunity.

During brucellosis, tuberculosis, leprosy, syphilis and other diseases with long duration the relative immunity coincides within a definite length of time with the

presence in the host of causative agents of infectious diseases. This type of immunity is named ***non-sterile*** (infection, *depression)* immunity.

During chronic diseases it has been possible to reveal for a long time the simultaneous presence in the body of the causative agent and relative immunity to repeated infection or to exacerbation of the existing infection. Specific defense reactions originate and develop simultaneously with the infectious process. Thus, during the stage of non-sterile immunity of different duration the development of all defense reactions without exception takes place, terminating in the formation of sterile immunity.

Thus, at first, infection and then post-infection immunity develops, and the phase of non-sterile immunity is replaced by the phase of sterile immunity. This general conformity is inherent to the infectious diseases of long duration (chronic), i.e. malaria, brucellosis, tuberculosis, etc.

Antitoxic immunity. In diseases the causative agents of which produce exotoxins certain tissues and organs are selectively infected. In the process of defense reactions evolution the body has developed the ability to render harmless not only the microbes, but their toxins. Toxins are rendered harmless mainly by neutralizing them with antitoxins. In the practice of immunization against diphtheria and tetanus antitoxic immunity is reproduced by introducing toxoids, and specific treatment of patients with diphtheria, tetanus, botulism and anaerobic infections is carried out with the corresponding antitoxic sera.

However, antitoxic immunity should not be reduced only to the reaction of neutralization. During infection with toxigenic microbes the host responds by producing defense mechanisms directed at the toxin and the causative agent.

Antiviral immunity. In many viral diseases (measles, rubella, yellow fever, smallpox, mumps, etc.) a sound immunity is built up, sometimes lifelong. Only after few viral infections (dengue fever) a weak immunity is produced.

Antiviral immunity provides protection against virus antigens - proteins and nucleic acids. Immunity against virus infections is manifested at all levels.

High-tension artificial immunity is reproduced to some virus diseases (smallpox, rabies, yellow fever, poliomyelitis, mumps, measles, Russian spring-summer encephalitis).

Antitumor immunity. Humans have a high-tension natural immunity to cancer and other malignant tumors. The regression of cancer cells to normal ones is encountered in some cases.

Antitumor immunity is displayed in 2 fomns, humoral with the production of antibodies and cellular with the participation of T-lymphocytes. Antibodies are produced under the effect of the antigens of DNA- or RNA-containing oncogenic viruses, antigens of tumors induced by carcinogens, antigens of transplants, embryonic antigens, etc. In some cases antitumor antibodies provide protection, in others they intensify growth of the tumor.

The thymus is an important organ in antitumor immunity. It ensures the elimination of cancer cells due to its ability to suppress the synthesis of nucleic acids and proteins. As distinct from transplantation immunity, in this type of immunity the forming foreign tumor tissue is not rejected.

Many types of cancer cells possess specific antigens capable of accomplishing defense without the participation of antibodies. They resemble protective antigens occurring in some infectious diseases of bacterial etiology (anthrax, plague, etc.).

Antiparasitic immunity. Insusceptibility to pathogenic parasites is characterized by a variety of mechanisms.

The production of immunity depends on the character of the localization of the parasite. Some of them localize in the tissues (trypanosomes, malarial plasmodia),

others in the lumen of the intestine *(Entamoeba histolytica)*, and others in the lumen of the intestine and in the tissues (intestinal balantidia, helminthes).

Antiparasitic immunity is brought about by the defense action of antibodies Ig E and an increased activity of phagocytes. Under the influence of antibodies the life processes in parasites are deeply disturbed, and then the parasites are dissolved. Phagocytes under the influence of opsonins absorb and digest minute parasites, while large parasites are immobilized in the tissues by the mutual action of many cells.

Community immunity. Besides the above mentioned forms of immunity there is the concept of community immunity (group, focal). This kind of immunity is created as a result of having had obvious or latent diseases, and also under the effect of a carrier state in definite foci of epidemic outbursts.

In most cases community immunity is post-infectious. In places with high morbidity of virus hepatitis, Russian springsummer encephalitis, and poliomyelitis the production of pronounced community immunity is also observed.

Community immunity is created not only as a result of epidemic outbursts, but also as planned immunimtion of the population.

Transplantation immunity. Transplantation immunity is explained by the fact that the grafted transplant differs genetically from the tissues and organs of the recipient. The donor's genome contains genes that are not present in the recipient, i.e. the introduced tissue is genetically heterologous.

The synthesis of transplantation antigens is determined by genetic structures which are called histocompatibility loci (H loci in animals and A/I-ILA in humans).

Transplantation immunity is due to cell reaction of the delayed hypersensitivity type, though antibodies appear in the recipient. The sensitized lymphocytes exert a cytopathogenic effect on cells which differ in I or more genes.

Non-specific Resistance

This form of defence which includes defensive properties is associated with phagocytosis, barrier function of the skin, mucous membranes, lymph nodes and other tissues and organs.

Phagocytosis. The most ancient form of immunity is phagocytosis. The phenomenon of phagocytosis is of great importance in defence reactions of heritable and acquired immunity.

For more than a quarter of a century, I. Metchnikoff accumulated facts confirming the defence role of phagocytosis during infection of vertebrate animals with pathogenic microbes. I. Metchnikoff subdivided those cells able to carry out phagocytosis into *microphages* and *macrophages.*

Microphages include granular leucocytes, neutrophils, eosinophils and basophils, of which only neutrophils have quite a marked ability for phagocytosis. Eosinophils and basophils are characterized by a weak phagocytic activity, although this problem has not yet been studied sufficiently.

Macrophages may be motile (monocytes of the blood, cells of the lymph nodes and spleen, polyblasts, histiocytes, etc.) or non-motile (reticular cells of the spleen, cells of the lymphatic tissue, endothelium of the blood vessels, etc.).

The process of phagocytosis consists of four phases.

The first phase involves the approach of the phagocyte to the microbe by means of a positive chemotaxis. Under the influence of the productsof the life activities of microbes excitation of the phagocytes occurs, which leads to a change in the surface tension of the cytoplasm, and gives the phagocytes amoeboid motility.

In the second phase adsorption of the microorganism on the surface of the phagocyte takes place.

The third phase is characterized by submergence of the microbe into the cytoplasm of the phagocyte.

The phagocyted bacteria perish under the bactericidal effect of the heightened hydrogen ion concentration due to an increase of lactic acid in the cytoplasm of the phagocytes.

In the fourth phase intracellular digestion of the engulfed microbes by the phagocytes takes place.

Factors which speed up phagocytosis include calcium and magnesium salts, the presence of electrolytes and antibodies (opsonins and bacteriotropins), histamine, pyrogenic substances capable of raising the temperature of the tissues and the entire organism.

Phagocytosis proceeds more vigorously in the immune than in the non-immune organism.

Toxins of bacteria, leucocidin, capsular material of bacteria inhibit phagocytosis.

Besides ***complete phagocytosis, incomplete phagocytosis*** is observed in certain diseases (gonorrhoea, leishmaniasis, tuberculosis, leprosy) in which microorganisms are absorbed by phagocytes, but do not perish, are not digested, and in some cases can reproduce.

Viruses are also digested in the macrophages of immune animals under the effect of the acid content of the vacuoles and the enzymes of the phagocytes though, unlike bacteria, viruses are intracellular parasites and are capable, to a great degree, of resisting phagocytosis.

The skin, mucous membranes and lymph nodes. In a normal, uninjured state, the skin not only is a true mechanical protective barrier, but a bactericidal factor. It has been established that the clean skin of a healthy person has a lethal action on a

number of microbes (haemolytic streptococcus, salmonellae of enteric fever and paratyphoid fever, colibacillus, *etc.).*

The mucous membranes of the eyes, nose, mouth, stomach and other organs have defence adaptations. Like the skin barrier, the mucous membranes perform antimicrobial function as a result of their impermeability to different microbes and the bactericidal action of their secretions.

In the tears, sputum, saliva, blood, milk, tissue fluids **lysozyme** is found. Microbes which have penetrated into the mucous membranes are continuously destroyed by the lysozyme. Bactericidal properties are not limited to the action of lysozyme.

There are other antibiotics produced by the organs and tissues, which are capable of inhibiting microbes. A special substance ***inhibin*** has been found in the saliva, and the antibiotic ***erythrin*** — in the erythrocytes.

Hyaluronic acid has *c*ertain significance of the physiological immunity. It inhibits the penetration of microbes into tissues and organs.

Besides the defence adaptations of the skin and mucous membranes, a large role is played in natural immunity by the *lymph nodes* in which the pathogenic microbes penetrating through the injured skin and mucous membranes are localized and rendered harmless.

A substance which has bactericidal properties with regard to a number of micro-organisms (causative agents of anthrax, tetanus, botulism, gas gangrene, and diphtheria, and staphylococci, pneumococci, bovine brucellae, etc) is **beta-lysin** which is a substance of a complex nature, a thermostable fraction of normal serum, decomposing at temperatures of 63-70°C or under the action of ultraviolet rays.

From human serum a fraction was isolated which is characterized by a bactericidal action in relation to diphtheria bacilli, and is not identical to beta-lysin.

Leukines are thermostable, bactericidal substances excreted by leucocytes. They disintegrate at a temperature of 75-80 °C. Leukines render harmless Gram-positive as well as Gram-negative bacteria.

C-reactive protein (the name is associated with C-polysaccharide of the type II *St pneumoniae)* having immunological properties was discovered m 1930 in the serum of patients with pneumococcal diseases. C-reactive protein is considered to be conjugated with reactive, defensive, non-specific natural processes It has also been found in the serum of patients with typhus fever, tuberculosis and other infections The component parts of urine, prostatic fluid, extracts from the liver, brain, spleen and other tissues and organs are characterized by bactericidal propertie.

Interferons are glycoproteins that block virus replication and exert many immunomodulating functions.

Alpha interferon (from leukocytes) and **beta interferon** (from fibroblasts) are induced by viruses (or double-stranded RNA) and have antiviral activity.

Gamma interferon is a lymphokine produced primarily by the Th-1 subset of helper T cells. It is one of the most potent activators of the phagocytic activity of macrophages, NK cells, and neutrophils, thereby enhancing their ability to kill microorganisms and tumor cells.

Properdin is a serum protein, an euglobulm, which plays an important part in immunity. The synthesis of complement, properdin, lysozyme, interferon, and other natural inhibitors is determined genetically, inherited, and belongs to the factors of species immunity.

Complement is a multicomponent system composed of many different proteins which belongs to non-specific defence.

There are two pathways for the activation of the complement. The **classic pathway** of complement activation is set in motion by antigen-antibody complexes,

whereas the **alternate pathway**, which is phylogenetically much older, is entirely independent of antigen-antibody reactions.

Instead, certain components are activated by the presence of a series of foreign substances, not the least of which are infecting bacteria and viruses.

The two pathways, however, have much in common, particularly the fact that their final membrane-attack components are identical.

Biologic Function of the Complement System.

Before the details of the classic pathway of complement activation are outlined, the biologic function of this system should be considered. This function can best be appreciated by noticing that after antibody has reacted with its antigen, it can do little more.

In other words, antibody might precipitate an antigen or, if the antigen is cellular, might cause agglutination but, with the exception of the neutralization of toxins or of virus infectivity, antibody alone is an ineffective means of protection against infection. Thus, for practical purposes, **the major function of an antibody is to recognize a foreign antigen and bind to it.** By doing so, it provides a site for phagocyte interaction and for the initiation of the reactions of the complement system.

It is the activation of this system that (1) leads to the lysis of foreign cells, (2) further enhances phagocytosis of invading microorganisms, and (3) causes local inflammation, stimulating the chemotactic activity of the host's leukocytes.

In addition, after activation of the system, interaction of one or more complement components with specific receptors on cell surfaces can result in

(1) enhancement of antibody-dependent cellular cytotoxicity (ADCC);

(2) increased oxidative metabolism;

(3) secretion of vasoactive amines and leukotrienes;

(4) secretion of monokines;

(5) stimulation of prostaglandin and thromboxane pathways;

(6) modulation of lymphocyte activation and antibody responses; and

(7) mobilization of leukocytes from the bone marrow.

The following sections are concerned with a step-by-step dissection of the component parts and reactions of this system and the role that it plays in the destruction of foreign cells.

Classic Pathway of Complement Activation. The operation of the complement system consists of a number of reactions, each of which activates the next reaction in the series. A primary event must occur, however, to initiate the reactions that eventually involve the many components of the complement system.

In the case of the classic pathway, the initiating event occurs when the first component of complement reacts with antigen-antibody complexes in which the antibody is either IgM or IgG. IgA, IgD, and IgE are not effective in activating complement.

Once initiated, the activation of the complement system may have various effects, depending on the type of foreign cell involved in the antigen-antibody reaction.

In the case of a gram-negative bacterium, the integrity of the cell membrane is destroyed, permitting the lysozyme-mediated lysis and death of the cell. Gram-positive organisms are not lysed, but the activation of complement by a gram-positive

cell and antibody results in the release of fragments of complement components that aid in phagocytosis by binding to the antigen, providing a receptor for the host leukocyte. In addition, many eukaryotic cells, such as virus-infected cells, are lysed by complement.

The complex reactions that produce all these effects can be divided into three series of reactions involving the complement system:

(1) the activation of the recognition unit,

(2) the assembly of the activation unit, and

(3) the assembly of the attack unit. Figure 1 summarizes the reactions of complement leading to the formation of the attack complex, and Figure 2 shows a schematic model of this series of reactions.

ACTIVATION OF RECOGNITION UNIT

$$C1 + Ab \rightarrow Ab\text{-}C1l\ (Ab\text{-}C1q\text{-}C1r\text{-}C1s) \rightarrow \boldsymbol{C1s}$$

ASSEMBLY OF ACTIVATION UNIT

$$C1s \downarrow C4 \rightarrow C4a + C4b$$

$$C1s \downarrow C2 \rightarrow C2a + C2b$$

$$C4b + C2a \rightarrow \textit{C4b2a}\ \ C3\ \text{Convertase}$$

$$\textit{C4b2a} \downarrow \textit{C3} \rightarrow \boldsymbol{C3a+C3b}$$

$$\boldsymbol{C4b2a} + C3a \rightarrow \boldsymbol{C4b2a3b}\ \ C5\ \text{Convertase}$$

ASSEMBLY OF MEMBRANE ATTACK COMPLEX

C4b2a3b

↓

$$C5 \rightarrow C5a + C5b$$

$$C5b+C6+C7 \rightarrow C5b67$$

$$C5b67 + C8 + C9 \rightarrow C5b6789_n$$

Alternate Pathway of Complement Activation. The alternate pathway of complement activation (the properdin pathway) does not require the presence of antibodies for initiation and, as a result, provides a mechanism of nonspecific resistance to infection.

Moreover, this pathway does not use C1, C4, or C2**,** which are the early reactants in the classic pathway of complement activation.

However, that the overall result of this pathway is the same as that of the classic pathway: C3 is split into C3a and C3b, and C5 is cleaved to form C5a and C5b, thus permitting the spontaneous formation of the C5b-9 membrane-attack complex.

The enzymes catalyzing these conversions are different from the C3 and C5 convertases described for the classic pathway of complement activation.

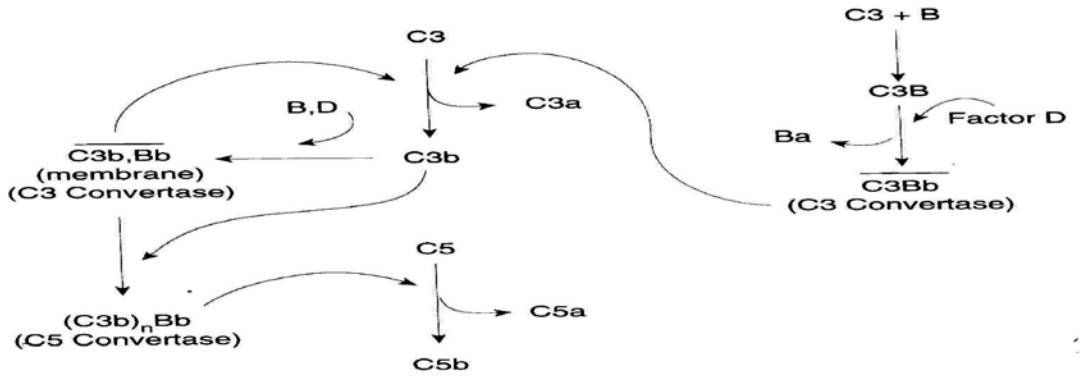

FIGURE. The alternate pathway of complement activation. Soluble C3 spontaneously interacts with factor B in solution. This complex is converted to a C3 convertase enzyme by factor D. The membrane-bound C3 convertase can be converted into C5 convertase by interaction with other C3b. The C5 convertase cleaves C5, and the resulting C5b initiates formation of the attack complex in the same way as described for the classic pathway.

NATURAL KILLER CELLS

Natural killer (NK) cells play an important role in the innate host defenses. They specialize in killing virus-infected cells and tumor cells by secreting cytotoxins (perforins) similar to those of cytotoxic T cell. They are called "natural killer" cells because they are active without prior exposure to the virus, are not enhanced by exposure, and are not specific for any virus.

They can kill without antibody, but antibody enhances their effectiveness, a process called antibody-dependent cellular cytotoxicity (ADCC). IL-12 and gamma interferon are potent activators of NK cells.

From 5 to 10% of peripheral lymphocytes are NK cells.

Many virus-infected cells and tumor cells display a significantly reduced amount of class I MHC proteins, and it is those cells that are recognized and killed by the NK cells.

Nonspecific host protection from microorganisms.

Relevance of the topic:

The complex processes and appearances in human body occur permanently. They are directed to support the functional integrity of the body, sustainability (permanence) of the chemical and cellular composition of the internal environment - that is, homeostasis.

Protecting the body against foreign agents occurs at two levels: non-specific resistance and specific immunity. The first - an older phylogenetic level consists of nonspecific factors of h host protection, which act against any foreign agent.

Nonspecific host defenses are the protective functions of the skin, mucous membranes, lymph nodes, gastric juice, hydrolytic enzymes; inhibitors production,

lysozyme, interferon and others; antagonistic properties of the human microflora; bactericidal properties of blood: complement, β-lysine, leykins, normal antibodies and other substances with bactericidal action; phagocytosis.

Mechanisms of nonspecific host protection function in the body permanently and cause inflammatory reaction in cases of massive or microbial (or) other destabilizing actions, the same - various causative agents.

Various diseases are observed when components of nonspecific host protection are violated.

Determination of quantitative changes, qualitative or functional state of various factors of nonspecific host defenses give possibility at value the state of the human immune system, help in the diagnosis, prevention and therapy of infectious and noninfectious diseases.

All this provides for the relevance of topic and directs a positive motivation to study.

Educational purpose:

• To study theof technique of determination of lysozyme in human serum by titration method.

• The complement titration test in serum provides for 100% hemolysis, consider features of this test, make conclusions.

• To study the phenomenon of incomplete phagocytosis on the smears that are made from urethral discharge in a patient with gonorrhea.

Terms	Definitions
Immunity	***Immunity*** – is a complex of processes and mechanisms that provide sustainability (permanence) of antigenic structure of an organism

	and its protection against infectious and other foreign agents for him.
Nonspecific host defenses	***Nonspecific factors*** of host defenses, as evolutionarily emerged before specific immunity, are non-specific mechanisms, physic-chemical, cellular, humoral, and physiological protective reaction that ensure the constancy of internal environment and restoration of disturbed functions of macroorganism. Non-specific resistance factors act quickly and constantly against all are the foreign agents. The non-specific resistance factors are: barrier function of the skin, mucous membranes, lymph nodes, bactericidal components of organism liquid (saliva, blood serum, etc), secretion function, fever, antagonistic properties of normal microbiota etc.
Lysozyme	***Lysozyme*** - is enzyme (acetylmuraminidase) that can destroy peptidopolysaccharide of grampositive bacteria cell wall, which consists from murein into the 90 %. Lysozyme is synthesized by macrophages and provides bactericidal properties of blood, saliva, and mucosa.
Complement	***Complement*** – is a complex set of blood proteins, which consists of 9 factions, each of which has a certain property. Complement is synthesized by liver cells and performs some functions: 1) causes lysis of microbes and other cells, 2) taks part in specific immunological reactions and virus neutralization; 3) intensifies phagocytosis, chemotaxis, and inflammation.
Properdine	***Properdine*** - is a high-(230 thousand Daltons) serum protein that

	participates in an alternative way of complement activation, eliminates some bacteria and viruses and stimulates phagocytosis.
Phagocytosis	***Phagocytosis*** - is the oldest form of host defenses, is an active absorption and digestion of live or killed microorganisms or other foreign particles by the cells. Two types of cells carry out phagocytic function: 1) microphags (neutrophils, eosinophils); 2) motile macrophages (monocytes, histiocytes, etc.) and motionless (cells of the spleen, lymphatic tissue, liver endotheliocytes, endothelium of blood vessels, etc.).
Uncomplete phagocytosis	***Uncomplete phagocytosis*** - is phagocytosis, in which microorganisms are absorbed by phagocytes, but not killed and digested, and sometimes multiply, causing the death of phagocytes.
Inflammatory reaction.	***The inflammatory reaction*** - is a reaction in which the tissues release various substances (leukotoxins, leukophenic factor, histamine, serotonin, etc.), under the action of which leukocytes activate, that do not allow bacteria to spread in tissue, blood and organs. Inflammation causes fever, acidosis and hypoxia, that make detrimental effect on microorganisms.
Interferons	***Interferons*** – make up a group of low-molecular-weight induced proteins that carry out control and regulatory functions aimed at preserving cellular homeostasis. The most important of these functions are antiviral, antitumor, immune-modulating, antibacterial and radioprotective. Interferon is synthesized by lymphocytes, leukocytes, fibroblasts, cells of the lymph nodes. Interferon is ubdivided into three types: interferon-α (from

	leukocytes), interferon-β (from fibroblasts) and interferon-γ (lymphocytic, or immune). Induction of interferon synthesis may be caused by viruses, bacteria, fungi, plant extracts, and synthetic compounds, various drugs, radiation, etc.
Acute phase proteins	***Acute phase proteins*** – make up a large group of proteins that are produced in the body during inflammatory responses after infection or injury, during ontogenecity, pregnancy and have antimicrobial action, promote phagocytosis, complement activation, the formation and elimination of inflammation. The bulk of acute phase proteins consist from C-reactive protein, serum amyloid A and P. Other acute phase proteins - are blood coagulate factors, metalic-binding proteins, protease inhibitors and some components of complement.
β -lysine	***Beta-lysine*** - is thermostable (destroyed at 65-70 ^{o}C) bactericidal factor, that is active against anaerobes and aerobic spore-forming bacteria.
Inhibitors of viral activity	***Inhibitors of viral activity***, this is the first humoral barrier that prevents virus contact with the susceptible cells. Thermostable inhibitors can inactivate infectious, toxic, haemagglutinate properties sensitive to inhibition of strains of viruses. Thermostable inhibitors can block connections of virus with the host cell receptors. People with high levels of inhibitors in the blood have a greater

	resistance to viral infections.
Cytokines	***Cytokines*** are the soluble mediators (like hormone) of host defense responses, both specific and nonspecific. The same cytokine can be produced by multiple cell types and can produce multiple effects on the same cell, and they can also act on many different cell types. Cytokines are the peptides or glucoproteins. As regulators of cytokines production there may be another cytokines, hormones, prostaglandins, antigens and many other agents, which can act to the cell.

Recommendations for design of the protocol
Scheme titration of lysozyme in saliva
Schematic representation of lysozyme titration

№ tube / Ingredients, ml.	Test tubes						control
	1	2	3	4	5	6	7
0,5% Isotonic sodium chloride solution	1,8 ↑	1	1	1	1	1	1
Patient's saliva	0,2 →	1 →	1 →	1 →	1 →	1 →	
Dilution of patient's saliva	1:10	1:20	1:40	1:80	1:160	1:320	
2 mlrd cell/ml M.lysodeikticus	1	1	1	1	1	1	1
Final dilution	1:20	1:40	1:80	1:160	1:320	1:640	
						↓ 1 ml	

Incubation in thermostate for 15 min at 45^{o}C (previous registration).
The final registration of the results is made after incubation of tubes at 37°C for 24 hours.
Titer of lysozyme - is that most of amount of serum dilution, which is able to give complete lysis of bacteria (M.lysodeikticus)

Schematic representation

of complement titration in human serum by 100% hemolysis.

№ tube / Ingredients, ml	1	2	3	4	5	6	7	8	9	10	11
Tested serum in 1:10 dilution	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9	1,0	1,2
Isotonic sodium chloride solution	1,4	1,3	1,2	1,1	1,0	0,9	0,8	0,7	0,6	0,5	0,3
Haemolytic system	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
In thermostate on 40-50 min at 37°C											
The results*	-H	-H	-H	+H	+H	+H	+H	+H	+H	+H	+H

Note: *-H – no hemolysis; +H –hemolysis is present.
Haemolytic system – a mixture of equal volumes of haemolytic serum (in 1:3 dilution) and 3 % suspension of erythrocytes of the sheep.

The mixture is incubated for 30 minutes at 37 °C erythrocytes sensibilization.

Titer of complement – is the smallest number of tested serum that provides complete hemolysis of added volume of sensibilized erythrocytes.

***Practical lesson* # 9**

Theme: **Immunity. Kinds and forms of immunity. Types of immune answer. Innate immunity. Nonspecific factors of organism defense against microbial agents. Cells and receptors of innate immunity.**

Questions of the learning.

1. Immunity. Central and peripheral organs of immune system.
2. Kinds and forms of immunity.
3. Types of immune answer. Innate immunity.
4. Nonspecific factors of organism defense against microbial agents.
5. Phagocytosis and tissues of the immune response, their functions.
6. Cells and receptors of innate immunity.

Independent work.

1. Explain the term « immunity » -

_____

_____

_____

2. Name the types of immunity by origin.

_____

_____

3. Name the types of acquired immunity.

_____

_____

_____

_____

4. Write the central and peripheral organs of immune system.

Central organs	**Peripheral organs**
_____	
_____	

_____
_____

_____
_____

5. Fill in the table

Difference between active and passive immunity

Active immunity	Passive immunity

6. Name the main groups of nonspecific factors of immunity and give examples:

1.

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2.

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_____
_____
_____

3.

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_____
_____
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7. What is the phagocytosis?

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_____
_____

8. Phases of the phagocytosis:

_____ _____
_____ _____
_____ _____
_____ _____

9. Draw the phagocytosis.

10. Cells and receptors of innate immunity.

_____
_____
_____
_____

Theme: **Adaptive immunity.T- and B-lymphocytes. Description of antigens. Presentation of antigens. Activating of lymphocytes. Antiinfectious immunity.** ***Immunological method of research*** **– serological reactions. Immunoglobulins.**

Serological reactions of agglutination and precipitation, immune lysis and compliment fixation test, their description and practical use. Coomb's test. Immunohematology. Application of monoclonal antibodies in immunocytochemical and immunohistochemical reactions.

Questions for the learning.

1. Antigens, their properties. Antigenic structure of the microbial cell.
2. Antibodies (immunoglobulins), their nature and structure.Classes of immunoglobulins, their characteristic.
3. Mechanism of interaction of antibodies with antigens. Specificity of immunological reactions.
4. Agglutination and precipitation assay, its mechanism, version, registration, practical value.
5. Complement fixation test: the mechanism, technique, ingredients, registration, practical values.
6. Complement: titer, working dose, value for human organisms. Classification of the immunoglobulins, their characteristic.
7. Application of monoclonal antibodies in immunocytochemical and immunohistochemical reactions.

Antigens. Their Nature and Properties.

The name antigens (from Greek worlds *anti* – against, *genos* – genus) is given to organic substances of a colloid structure (proteins and different protein complexes in combination with lipids or polysaccharides) which upon injection into the body (subcutaneously, intracutaneously, cutaneously, into the mucous membranes, intramuscularly, intraperitoneally, intravenously and orally) are capable of causing the production of antibodies and reacting specifically with them.

Antigens are microbes, their toxins, protein (albumen) and other substances.

Signs of Antigens

1. Heterogenity in the respect of genetics;
2. Antigenicity is the ability to cause formation of antibodies;
3. Immunogenity is the ability to create immunity;
4. Specificity, antigens abilities which differ from each other;
5. Antigens ability to interact with antibodies;
6. Antigen and antibody molecules are complementary; they suit each other as a key suits a padlock.

Antigens are complete, incomplete (hapten), semihaptens, autoantigens (isoantigens) and heteroantigens.

1. **Complete antigens,** substances causing antibodies (immunoglobulins) producing and reacting with them. A complete antigen consists of two parts: high molecular carrier (protein, polysaccharid) carrying out antigen function and low molecular determinant grouping (acid radicals, amino acids, dipeptids and others), responsible for antigen specificity.
2. **Incomplete antigens** (hapten) don't cause specific antibodies formation (they are of low molecular weight) but they are able to enter into reactions with ready antibodies. Hapten can be combined with protein to get complete antigen. Lipids, carbohydrates, vitamins are referred to haptens.
3. **Semihaptens** don't cause producing antibodies but they combine with proteins in a human organism and turn into (transform) complete antigens. They are antibiotics, sulphamid drugs and other drugs.
4. **Autoantigens** are proteins of an organism's tissues, but they are changed due to the effect of drugs, microbes, toxins and other things. They are eye crystalline, brain, spermatozoa, and parachute like glands.
5. **Heteroantigens** are antigens, which are found in different kinds of animals. Heteroantigens are found out in a human being and in some kinds of bacteria. For

example, pathogenic organisms of plague and human erythrocytes of 0 blood group have common antigens.

Bacterial cell is a compound complex of antigens. Some antigens are similar in close types of microorganisms.

They distinguish group, specific and type specificity of antigens.

Group specificity is inherent in a group of individuals, which have common genetic connections.

Specific – representatives of one species differ from another. Type specificity is caused by the presence of typical substances, which are specific for a definite type. These antigens differences are of practical significance when defining a group, species and a type of microorganism.

Antigenic structure of the microbial cell

Bacteria are a complex of antigens which include highly molecular compounds of a protein nature and biologically active specific polysaccharides.

Different structures of cells contain them and they perform definite functions.

O - antigen (somatic) is available in a cover (coat) of a bacterium cell, it plays the main part in formation of immunity against microbes.

H - antigen (flagellar) is available in flagella of mobile bacteria.

K - antigen (capsular) is found in capsule of a bacterium.

Vi - antigen is an antigen of pathogenecity, it raises virulence of bacteria.

There are surface and core antigens of virus. Products isolated by a bacterium cell as well as ferments are antigens.

ANTIBODIES - IMMUNOGLOBULINS

Antibody is defined as humoral substance (gamma-globulin) produced in response to an antigenic stimulus. It served as protective agent against organisms. Antibodies are found in serum , lymph and other body fluids.

Sera having high antibody levels following infection or immunization is called immune sera. Antibodies are :

1. Protein in nature.
2. Formed in response to antigenic stimulation
3. React with corresponding antigen in a specific and observable manner.

The antibody molecular is chemically indistinguishable from normal gammaglobulin. Globulin is a very complex mixture of molecules consisting of closely related proteins.

Now the term immunoglobulin is used to describe these closely related proteins.

Immunoglobulins are synthesized by plasma cells and also by lymphocytes. Immunoglobulins make 20 to25% of the total serum proteins. The term immunoglobulins is structural and chemical concept while, antibody is biological and functional concept.

All antibodies are immunoglobulis but all immunoglobulins may not be antibodies.

Based ao their size, carbohydrate contents and amino acid analysis, five groups of immunoglobulins have been distinguished. IgG, IgA, IgM, IgD and IgE.

Structure of Immunoglobulin

Immunoglobulins are glycoproteins. Each molecule consisting of two pairs of polypeptide chains of different size held together by disulphide bonds (S-S). The

smaller chains are called light (L) chains and larger ones heavy (H chains).The H chains are structurally and antigenetically distinct for each class and designed by ***Greek letter as follows:***

IgG	**Gamma**
IgA	**Alpha**
IgM	**Mu**
IgD	**Delta**
IgE	**Epsilon**

The L chains are similar in all classes.

Fc fragment of H chain determines biological properties of immunoglobulins molecules like, complement fixation, placental transfer, skin fixation attachment to phagocytic cells, degranulation of mast cells and catabolic rate.

The function of ***Fd fragment of H chain*** is unknown.

Fab: It is half of heavy chain and one light chain.It acts as antigen binding fragment.

Immunoglobulin classes

IgG. It is a major serum immunoglobulin. It's molocular weight of 150000.It is distributed equally between intravascular and extravascular compartments. It passes through placenta and provides natural passive immunity to new born. It produces passive cutaneous anaphylaxis.

It Participated in immunological reactions like precipitation, complement fixation, neutralization of toxin and viruses.

IgA. It is fast moving gamma globulin. It constitutes 10% of total serum globulin. It molecular weight is 160000. It is found in high concentration in colostrum, tear, bile, saliva, intestinal and nasal secretion.

Its amount is greatly increased in cases of multiple myeloma.

It does not pass through placenta. IgA does not fix complement but activate alternate complement pathway. It promotes phagocytosus and intracellular killing of organisms.

IgA found in secretions contain additional unit called transport (T) or secretary (S) piece. T piece is synthesized in epithelial cells of gland, intestines and respiratory tract.

It is attached to IgA molecuie during transport across the cells. T piece links two IgA molecule at Fc portion. J chain is also found in IgA. J chain as synthesized by lymphoid cell.

IgM. It is also called macroglobulin. It constitute 5 to10% serum globulin. Molecular weight is 900000 to 1000000. Mostly it is intravascular.

IgM appears earlier in primary response and IgG is produced later. Its half life is 5 days and it fixed complement. It does not pass through placenta.

IgM is more efficient in agglutination, cytotoxic and cytolytic reaction. Its deficiency is often assotiated with septicaemia.

IgD. It is mostly intravascular. It has half life of 3 days. It seems likely that IgD may function as mutually interacting antigen receptor for the control of lymphocyte activation and suppression. It is very sensitive to proteolytic degrdation.

IgE .It is reaginic antibody responsible for immediate hypersensitive reactions. It has molecular weight of 19000. Its half life is 2 days.

It has affinity to surface of tissue cells (particularly mast cells) of the same species. It does not pass through placenta or fix complement. It is mostly intravascular in distribution.

Normally it is found in traces in serum. Elevated levels are seen in atopic condition like asthma, hey fever and eczema.

Children having parasitic infection in intestine show elevated levels of IgE.

Serological tests

Relevance of the topic:

Methods of treatment of infectious disease are determined by biological characteristics of causative agent.

That is, the doctor should not only put a clinical diagnosis, but also determine which organism caused the infection.

Etiological diagnosis of many infectious diseases is based on an isolation of pure culture of causative agent and its identification.

The identification of most bacteria and viruses is based on the determination of specific antigens, we use serological tests for this task.

The purpose of this investigation is the serological identification. In addition, the diagnosis of infectious of diseases can be based on identification of specific microbial antigens directly in patient specimens (blood, spinal fluid, urine, etc..), especially, if we can not cultivate and make pathogen's identification by other methods.

On the other side, the immune response manifested, in particular, by development of specific antibodies to each species of causative agent.

This allows us to put the etiological diagnosis by detection of these antibodies. For this purpose we carry out serological tests for serological diagnosis.

Specific objectives:

- To familiarize with the purpose of using serological tests (ST).
- To examine the medicine, used for serological identification.
- To familiarize with the features of agglutination and precipitation tests.
- To master glass agglutination test and direct agglutination test (AT).
- To learn the technique of precipitation test (PT).

A list of key terms, parameters, characteristics

that a student should learn for the lesson:

Terms	Definitions
Serological tests	The reaction of a specific interaction between antigens (Ag) and antibodies (Ab).
Agglutination test	Agglutination – is clumping of corpuscular Ag (bacteria, erythocytes) under the action of specific antibodies in the presence of electrolyte. Agglutination is a method of founding and quantitate detection Ag or Ab, based on their ability to form visible conglomerates.
Precipitation test	Precipitation is sedimentation of dispersed or soluble molecular Ag under the action of specific immune serum.
Serological identification	Serological identification - serological tests for detection (identification) unknown Ag using familiar Ab
The immune diagnostic serum	Immune diagnostic serum (IDS) - a standard preparation, which contains antibody to a definite group of microbes. It is used for serological tests.

REATIONS OF IMMUNITY

Antigen-antibody rections are useful in laboratory diagnosis of various diseases, and in the identification of infectious agents in epidemiological survey. Antigen antibody reactions in vitro are called serological rections.

Features of Antigen- Antibody Reactions

1. The reaction is highly specific.
2. Entire molecules react and not fragment.
3. There is no denaturation of antigen or antibody during reactions.

4. Combination occurs at surface and hence surface antigen are immunologically relevant.
5. The combination is firm but reversible. It is influenced by affinity or avidity. Affinity is intensity of attraction between antigen and antibody molecules. Avidity is strength of the bond after the formation of antigen antibody complex.
6. Both antigen and antibody participate in the formation of the agglutinates or precipitates.
7. Antigen and antibody may combine in varyingproportions.

PRECIPITATION

When a soluble antigen combines with its antibody in presence of electrolytes (NaCl) at a suitable temperature and pH the antigen antibody complex forms insoluble precipitate.

Uses of precipitation Reaction

1. Identification of bacteria e.g., detection of group specific polysaccharides substance in strestococci
2. Identification of antigen component of bacteria in infected animal tissue e.g., Bacillus anthracis (Ascoli test).
3. Standartizatuin of toxin and antitoxins.
4. Demonstration of antibody in serum e.g., Kahn's test for diagnosis of syphilis.
5. Medicolegal serology for detection of blood, serum etc.

Mechanism of precipitation:

Lattice hyporthesis explains it. Multivalent antigen combine with bivalent antibodyin varying proportions, depending on antigen antibody ratio in reacting mixture.

Precipitation results whenlarge lattice is formed consisting of alternating antigen and antibody molecules. This is possible only in the zone of equivalence. In zone of antigen and antibody excess lattice does not enlarge asvalensy of antigen and antibody is fully satisfied.

AGGLUTINATION REACTIONS

When a particulate antigenis mixed with it's antibody in presence of electrolytes at a suitable temperature and pH, then the particles are clumped or agglutinated. It is more sensitive than precipitation for the detestion of antibodies.

Uses

1. Identificetion of bacteria e.g./serotyping of salmonella and shigella with known antisera.
2. Serological diagnosis of infection e.g., Widal test for typhoid etc.
3. Hemagglutination test

Technique of Agglutination test

Direct aagglutination test:

Microagglutination: It is carried on a clean slide by mixing a drop of antiserum and antigen suspension. Reaction occurs immediately. It is used for detecting bacterial antigen, blood grouping and typing etc.

*Macroagglutination:*It is carried out as quantitative test to estimate the titre of antibody and to confirm the result of microagglutination. Following type of agglutination are observed with bacterial antigen:

1. Flagellar antigen or "H" type of agglutination is seen when a formalized suspension of motile bacteria is treated with antiserum . It forms floccular, snowy flakes like deposit. Agglutination appears 2 to 4 hours after incubation at 52^0C.

2. Somatic “O” type of agglutination occurs when heat killed or alcohol treated suspension of bacteria is treated with homologous antiserum. The agglutination is compact with fine granulation.

The reaction appears 18 to 24 hours after incubation at 37^0C.

4. Vi- agglutination is similar to O agglutination and occurs slowly at 37^0 C.

Scheme of agglutination test for finding antibodies in patient serum.

Ingredient (in ml)	Number of the test tube						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Isotonic sodium chloride solution	1,0	1,0	1,0	1,0	1,0	1,0	---
The patient's serum in a 1: 25 dilution (0,1 ml serum + 2,4 ml Isotonic sodium chloride solution)	1,0	1,0	1,0	1,0	1,0	in disinfect solution	1,0
diagnosticum	0,1	0,1	0,1	0,1	0,1	0,1	---
The obtained dilution of the serum	1:50	1:100	1:200	1:400	1:800	antigen control	serum control

The previous results of reaction are registered after 2 hours,

and final – after 18-20 hours of thermostat incubation at 37^0C.

Recommendations for the design of the protocol.

The glass-agglutination test.

Materials:

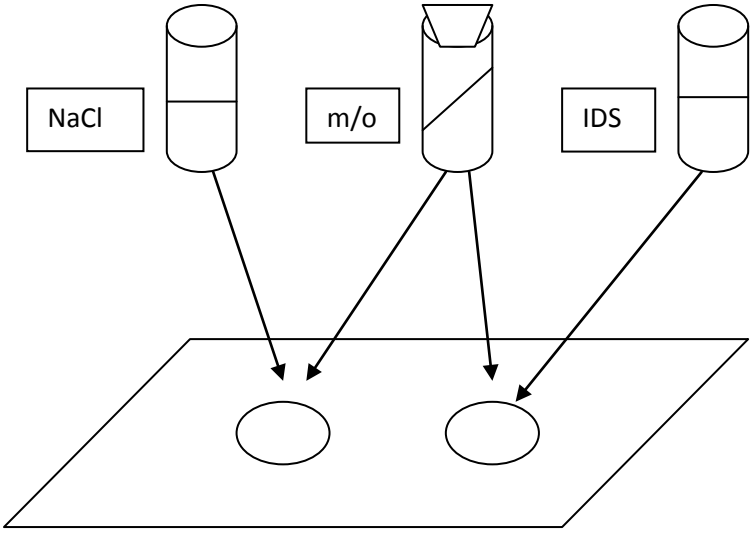
• Culture of bacteria

• Adsorbed immune diagnostic serum (IDS) for glass-agglutination test

• 0,5% solution of NaCl

• Glass for smears, bacteriological loop

The procedur:



The results of test: Positive test: is manifested by clumping formation.

The direct agglutination test for serological identification of bacteria culture

Materials:

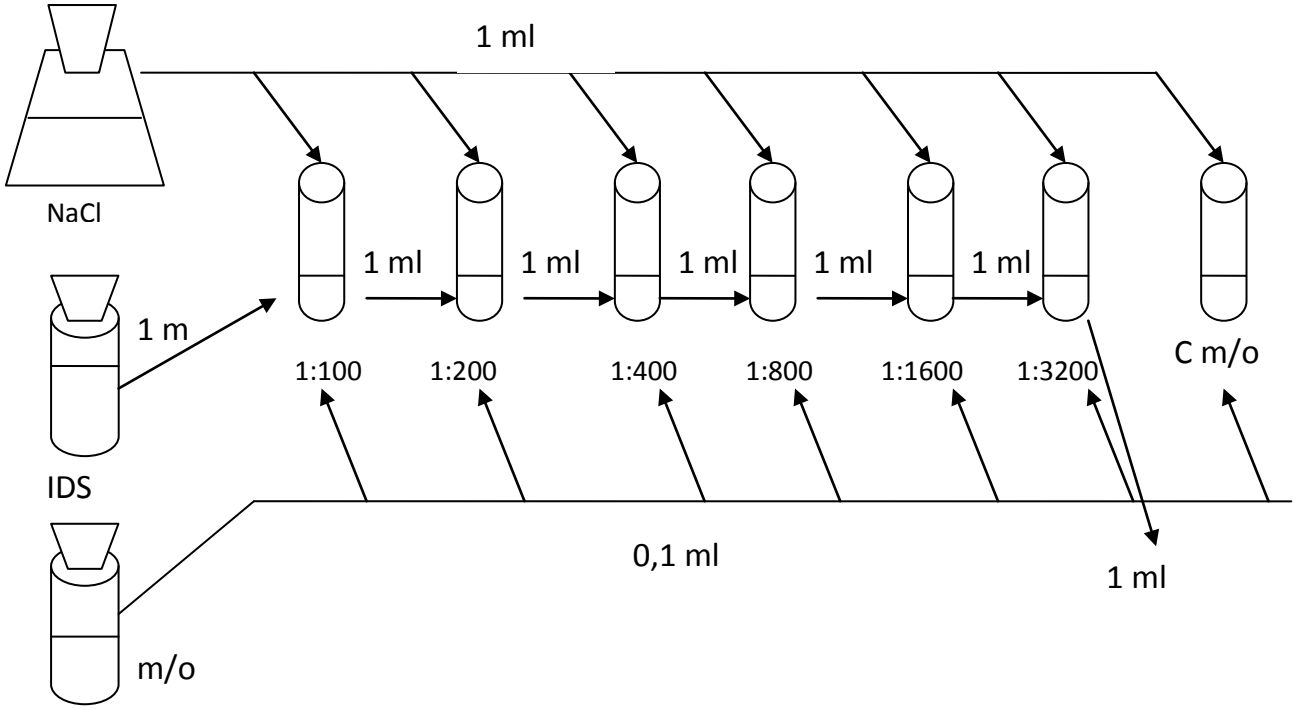
• Bacteria culture (suspension)

• Immune-diagnostic serum in the working dilution (1:50), titer - 1:3200

• 0,5% solution of NaCl

• Test tubes, pipettes

The procedure:



The results of test: Positive test – is manifested by agglutinate formation;

Previous registration - after 2 hours, final regisration – after 18-24 hours.

COOMB'S TEST

It is used for the detection of incomplete antibodies (non agglutinating anti Rh antibody), brucella, shigella and salmonella antigen.

Sera containing incomplete anti Rh antibodies is mixed with Rh positive red cells. Antibody globulin coats the surface are washed free of all unattached proteins are treated with rabbit anti serum against human gamma globulin (Coomb serum).

The cells are agglutinated. This is the principle of Coomb's test.

COMPLEMENT FIXATION TEST (CFT)

This is very sensitive test and is capable of detecting o.04 ug of antibody nitrogen and 0.1ug of antigen. It is used for serological diagnosis of diseases:

1. Bacterial diseases e.g., gonorrhoea, brucellosis.

2. Spirochaetal diseases e.g., syphilis (Wassermann reaction) etc.
3. Rickettsial diseases e.g., thyphus fever.
4. Viral diseases like lymphogranuloma venereum
5. Parasitic diseases e.g., kala azar, hydatid cyst, amoebiasis.

Principle: The ability of antigen antibody complex to fix complement.

Interpretation:**If** complement has been used up, there would not be hemolysis. It means antigen antibody reaction has taken place. Test is reposted as positive.

If sensitized RBC are lysed it means complement has not been fixed and test is reported as negative.

Term	**Definition**
1	2
Lyses reaction	Lyses reaction is antigen dissolving under the action of antibody in the presence of complement. Fresh human immune serum can do lyses, because contain antibodies and complement. If serum was heated or stored some time, lyses can be only with adding of complement.
Immune haemolysis reaction	The hamolysins (specific protective antibodies) are formed in the animal's blood serum after animal's immunization by erythrocytes. The haemolysins can destroy connection haemoglobin with erythrocytes' stroma in the presence of complement, and provide haemolysis reaction. The immune serum that contains haemolysins has the name of haemolytic serum. Such serums are produced by special laboratories.
The power of haemolytic serum	The power of haemolytic serum measured in titers – this is the maximum haemolytic serum dilution in volume 0,5 ml, that lead to fool haemolysis of 0,5 ml 3% sheep erythrocytes in the presence of 0,5 ml complement (in 1:10 dilution), and tubes incubation for 1 hour at 37^0 C.

Indirect haemagglutination (IHA) reaction	The essence of indirect haemagglutination (IHA) reaction is the ability sheep erythrocytes (or other spacies of animal) to absorbe Ag on its surface, and becom “sensitive” (sensibilized) to the corresponding immune serum. Sensibilized red blood cells stick together under the influence of specific antibody and form sediment (haeamagglutinate) at the bottom of the tube. We can detecte a minimum number of antibodies with IHA reaction because of high specifity and sensitivity of this reaction.
Complement system	Complement system – are the group of serum proteins, which after their activation are converted to effector molecules that lead to the development of inflammation (C3a,C, C4a), phagocytosis (C3b) and destruction of cells (C6-9). The complement proteins are involved in the development of inflammatory reactions, reactions opsonizations and lysis of cell membranes.
Diagnosticums	Diagnosticums – are the standard antigens. They can be suspensions of live bacteria (or inactivated), viruses or their antigens in isotonic solution. Diagnosticums are used for serological diagnosis of infectious diseases.

Complement fixation (CF) test for detection of antibodies in the test serum.

Complement fixation test is based on the ability of a specific complex antigen + antibody adsorb (bind) a complement.

As the process of complement fixation is not visuale, hemolytic system (sheep erythrocyte +hemolytic serum) is used as an indicator, which shows the effects of the reaction between antigen and antibody.

If the antigen and antibody are homologous with each other, then this complex binds complement and hemolysis does not occur, and if the complex does not bind with complement, the hemolysis follows.

CF test belongs to the complex serological tests and for its implementation there should be not less than 5 ingredients: antigen, antibody and complement (first system), sheep red blood cells and their homologous hemolytic serum.

Scheme of complement fixation (CF) test

for detection of antibodies in the test serum

Number of tube / Ingredients (in ml)	Test tube	Control tubes				
	1	2	3	4	5	6
Serum assayed in dilutions 1:10	0,5	---	0,5	0,5	---	---
Antigen (working dose)	0,5	0,5	---	0,5	0,5	0,5
Complement (working dose)	0,5	0,5	0,5	---	0,5	0,5
Positive serum (1:10)	---	---	---	---	0,5	---
Negative serum (1:10)	---	---	---	---	---	0,5
Isotonic sodium chloride solution	---	0,5	0,5	0,5	---	---
Incubation at 37 °C for 2 h						
hemolytic system	1,0	1,0	1,0	1,0	1,0	1,0
Incubation at 37 ºC for 45 min						

IMMUNOFLUORESCENCE

Fluorescence is the property of absorbing light rays of one wave length and emitting rays with different wave length.

Serological reactions employing tagged are used to detect minute amounts of weakly active antigen or antibodies.

The method is suitable for only qualitative reactions. The fluorescent antibody technique is used for:

1. Rapid serological diagnosis of number of bacteria.
2. Detection of antitoxoplasma antibody.
3. Demonstration of leptospira in human and animal muscles.
4. Detection of viruses in cells.

The various modification of fluorescent methods are as under:

a) ***Direct method****:* It is commonly used for detection of antigen by using of asingle layer of fluorescent labelled antibody.

b) ***Indirect method (double layer technique):*** It is used by treating a slide smear of the antigen with specific unlabelled serum. The preparation is thoroughly washed and is treated with fluorescence labeled gammaglobulin against the human serum.

c) ***Sandwitch technique*** is used for detection of antibody in tissue . tissue section is treated with dilute solution of antigen.
After washing (remove excess of antigen) section is exposed to fluorescein labeled antibodies.

Practical lesson # 10

Theme: **Adaptive immunity.T- and B-lymphocytes. Description of antigens. Presentation of antigens. Activating of lymphocytes. Antiinfectious immunity. *Immunological method of research* – serological reactions. Immunoglobulins. Serological reactions of agglutination and precipitation, immune lysis and compliment fixation test, their description and practical use. Coomb's test. Immunohematology. Application of monoclonal antibodies in immunocytochemical and immunohistochemical reactions.**

Questions for the learning.

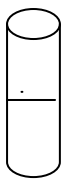
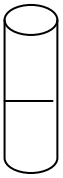
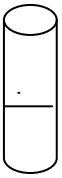
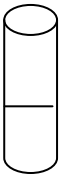
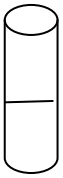
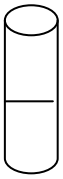
1. Antigens, their properties. Antigenic structure of the microbial cell.
2. Antibodies (immunoglobulins), their nature and structure.Classes of immunoglobulins, their characteristic.
3. Mechanism of interaction of antibodies with antigens. Specificity of immunological reactions.
4. Agglutination and precipitation assay, its mechanism, version, registration, practical value.
5. Complement fixation test: the mechanism, technique, ingredients, registration, practical values.
6. Complement: titer, working dose, value for human organisms. Classification of the immunoglobulins, their characteristic.
7. Application of monoclonal antibodies in immunocytochemical and immunohistochemical reactions.

Perform and read tubes agglutination assay. Determine the titer of reaction.

Agglutination assay

Ingredient	Number of the test tube						
	1	2	3	4	5	6 antigen control	7 serum control
0.9% NaCl solution, ml							
The patient's serum (1:50),ml							
Serum dilution							
Diagnosticum or antigen, drops							

Incubation at 37 º C for 2 hours in the thermostate.

Results							

The titer[1] of agglutination assay _____

Conclusion on agglutination assay_____

[1] The titer of the AB is the maximum dilution of the serum inducing the clear agglutination of the microbes.

1 : 200 is the diagnostic titer for the majority of the infectious diseases.

1 : 100 is doubtful titer. It's necessary to question the patient, whether he has been ill already or vaccinated.

<u>The mechanism of agglutination assay.</u>

1. **Stage specific invisible.**

2. **Stage specific visible.**

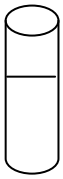
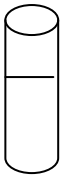
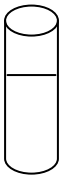
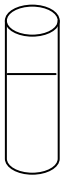
_____
_____
_____
_____
_____

Variances of agglutination reaction:

_____
_____
_____
_____

1. **Fill in the table.**

Precipitation assay

Ingredient	Number of the test tube			
	1	2	3	4 Experimental test tube
Normal serum, ml				
Immune serum, ml				
Positive extract, ml				
Extract to be tested, ml				
Results				

Conclusion on precipitation test

_____

_____

Variances of precipitation test:

_____

_____

_____

_____

3. Properties of Ag – AB complex:

1._____

2._____

3._____

4._____

4. Explain the term “antigen” and name its main properties.

_____
_____
_____
_____
_____
_____

5. Antigenic structure of the microbial cell.

_____
_____
_____

6. Explain the term “antibodies”.

_____
_____
_____

7. Name immunoglobulin classes and their brief characteristic.

#_____
_____
_____

#_____
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#_____
_____
_____

#_____
_____
_____

8. What the type of immunoglobulin is the first detected during in the infectious diseases?_____
_____

9. Name cells synthesized immunoglobulins.
_____

10. Fill in the table. **Complement fixation test (CFT)**

Ingredients, ml	**Number of the test tube**			
	1	**2**	**3**	**4**
Assayed serum (patient's serum)				
Antigen				
Complement				
0.9% NaCl solution				
Incubation at 37 ° C for 45 minutes in the thermostate.				
Hemolytic system (hemolytic serum in triple titer + 3% suspension of sheep erythrocutes)				
Incubation at 37 ° C for 30 minutes in the thermostate.				
Results				

Conclusion

_____

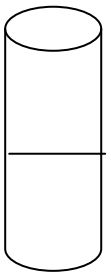
_____

CFT used for serological diagnosis of diseases:

1. _____
2. _____
3. _____
4. _____

The mechanism of complement fixation test

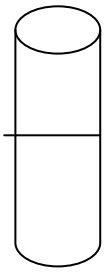
1.



Result -

_____

2.



Result -

_____

10. What is complement? Name properties of the complement.

_____
_____

_____

11. Name functions of the complement._____

_____
_____

12. Complement activation:

_____

_____

13. Application of monoclonal antibodies in immunocytochemical and immunohistochemical reactions.

_____
_____
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_____
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Theme: **Immune serums and immunoglobulins. Reaction of flocullation (neutralization). Hypersensitivity. Autoimmune phenomena. Principles of the use of antibodies as medical, preventive and diagnostic preparations.**

***<u>Questions for the learning</u>*.**

1. Assigning of immune serums, classification, application, reception.
2. Antitoxins, their characteristic. Antitoxic sera. Merits of E. Bering. Diagnostic serums, perception and application. Normal sera of the blood.
3. Gammaglobulines, structure, reception, application, mechanism of action.

4. Hypersensitivity. Types of hypersensitivity reactions and their mechanism of development.
5. Anaphylaxis. Serum sickness and its prevention.
6. Allergic reactions for immunodiagnosis of infectious diseases. Skin test (intradermal) and its diagnostic importanance.
7. Autoimmune phenomena.
8. Principles of the use of antibodies as medical, preventive and diagnostic preparations.

Immune sera are the biological preparations containing ready specific antibodies, injection of which in organism causes immediate obtaining of passive humoral immunity able to defend organism against intoxication or infection.

Besides immune antibodies, immune sera also contain another proteins of organism, from which the serum was obtained.

On the direction of their action all sera are divided on ***antitoxic, antimicrobial, antiviral.***

On application all sera are divided on sera for treatment and prophylaxis and diagnostic ones.

Sera for treatment. In 1886 E. von Bering found antibodies in the blood of porpoises, immunized with sublethal doses of diphtherin. In 1896 von Bering used sheep serum for diphtheria treatment.

Bering was awarded Nobel Prize for the creating of new treatment method and for the successes in diphtheria treatment.

Then appeared that horses are the best producers of serum. Immune sera from these animals are obtained by multiple immunization (hyperimmunization), during that immunization is performed by toxoid in increased doses.

Immunization is stopped when the animal ceases to increase the antibodies titer on repeated injection of antigen. After the completing the immunization (in 10-15 days) it is possible to take the blood. Blood serves for the obtaining of serum, which is purified, preserved, checked on sterility, harmless, protein concentration, transparency.

Serum purification from ballast substances is performed by dialysis and fermentation.

Unit of serum activity measurement is antititoxic unit. One antitoxic unit is minimal serum amount which neutralizes certain amount of toxin DLM for animals of certain kind and weight. Immune sera are used for specific treatment and urgent prophylaxis.

The chief mechanism of their action is antibodies neutralize bacteria,

viruses, toxins.

As horse antitoxic sera are heterogeneous they can cause allergic reactions on alien horse protein especially at repeated injection. In this connection the injection of treatment sera is performed by Bezredko method.

There are often applied diphtheric, botulinus, tetanus antitoxic treatment sera.

Diagnostic sera are widely used for serological diagnostic of many bacterial and

viral infections. Diagnostic O-, K-,H-,Vi-sera exist which are used in reactions of agglutination, precipitation and indirect hemagglutination. Diagnostic sera can be mono- and polyvalent.

Immunoglobulins

Immunoglobulins are biologic preparations which contain specific antibodies (immunoglobulins) injection of which to the organism induces to immediate occurrence of passive artificial humoral immunity able to protect organism from intoxication or infection.

As against from treatment sera, preparations of immunoglobulins do not contain ballast protein substances which are able to induce allergic reactions. Immunoglobulins are divided on preparations for treatment, diagnostic, antibacterial (against whooping cough and tetanus) and antiviral (against rabies) ones.

The raw for the preparation of normal human immunoglobulin can be donors blood plasma pool, placental blood serum pool.

The extraction of immunoglobulins from blood pool is performed by means of salting, immune sorption and dialysis. Serum of specially immunized donors is used for emergency prophylaxis and treatment of tetanus, Russian spring-summe (tick) encephalitis, influenza, rubella, whooping cough et al.

Heterologous preparations of immunoglobulins (for treatment and diagnostics) with high titers of specific antibodies can be prepared from serum of hyperimmunized animals.

Prospects of further perfection of preparations for the creation of passive immunity are connected with obtaining of monoclonal antibodies from human cells hybridoma. Such preparations will possess the most purposeful action at minimal risk of complications.

Immune sera and immunoglobulins

Immune sera and immunoglobulins for treatment and prophylactic	**Immune sera for diagnostic**
<u>Antitoxic and antibacterial</u> Diphtheria sera; Tetanus sera and tetanus immune globulin; Gas gangrene mono-and polyvalent serum; Staphylococcal immunoglobulin; Antianthrax globulin;	• **For identification of bacterial infections** agglutination precipitation lyzise • **For virus identification** Virus neutralisation Complement fixation Antihaemagglutination

• **Antiviral** Measles immunoglobulin Rabies gamma -globulin Influenza immunoglobulin Smallpox immunoglobulin Immunoglobulin for tick-borne encephalitis virus	

Immunomodulators

Immunomodulators are chemical and biological agents which stimulate, depress and regulate immune reactions. Homologous immunomodulators are secreted in the organism, e. g. cytokins, interleukins, the factor of tumoral necrosis. Heterologic immunomodulators are chemical substances, e. g. levamizol (decaris), levakadin, cyclosporin A. Immunomodulators are divided into three groups: immunostimulators, immunodepressants and preparations of substitutional therapy. Immunomodulators are administered at primary and secondary immunodeficiencies, malignant diseases.

Immunobiological preparations are those which influence the immune system, affect through the immune system and the principle of their action is based on immunologic reactions.

Immunobiological preparations are various by nature, by origin and by the way of receiving and using them.

HYPERSENSITIVITY

History: Immune response was thought to be protective. It may be activated by milk protein. Later on Portier and Richet (1902) showed that immune responses possess harmful effects by administering sea anemones to dogs.

First dose did not produce any harmful effect but second dose made the dog ill and he died within few minutes. Theobald Smith in 1904 observed same response in guinea pig using non toxic antigen.

With horse antisera and rabbit antisera used in human diseases harmful effects of immune response became obvious.

Von Pirquet (1906) suggested the term allergy which is an altered response of tissue to repeated contacts with antigenic agents.

Allergy was thought to include:

(a) Hypersensitivity (increased susceptibility).

(b) Immunity (increased resistance).

HYPERSENSITIVITY is defined as altered state induced by an antigen in which pathological reaction can be subsequently elicited by that antigen or by structurally similar substance.

In hypersensitivity focus of attention is what happens to host as a result of immune reaction. In immunity focus of attention is antigen (killing, neutralization of toxin).

Mechanism: The reactions that appear within minutes are mediated by freely diffusible antibody molecules (immediate type).

The other type is slow evolving responses that are mediated by sensitized "T" lymphocytes. This is cell mediated hypersensitivity (CMI i.e., delayed type).

Classification

(A) On the basis of time required for sensitized host to develop conical reaction upon reexposure to the antigen, Chase classified them as:

(i) Immediate reaction.

(ii) Delayed reaction.

The differences between them are:

	Immediate reaction	*Delayed reaction*
(1)	Appears and recedes rapidly.	Appears slowly and lasts longer.
(2)	Induced by antigen or hapten by any route.	Induced by infection, injection of antigen with Freund's adjuvant or by skin contact.
(3)	Circulating antibodies present (antibody mediated reaction)	Cell mediated reaction and not and responsible for reaction , antibody mediated.
(4)	Passive transfer possible with serum.	Transfer possible by lymphocytes or transfer factor.
(5)	Desensitization easy but short	It is difficult but long lasting, lived.
(6)	Lesions are acute exudation and fat necrosis.	Mononuclear cell collection around blood vessels.
(7)	Wheal and flare with maximum diameter in 6 hours.	Erythema in duration with maximum diameter in 24 to 48 hours.

(B) *Coomb's and Cell classification (1969):* They have classified hypersensitivity reaction into *5* types on the basis of different mechanisms of pathogenesis. It is widely used:

Type I: Anaphylactic, reagin dependent e.g., anaphylaxis, atopy etc. IgG, IgE and histamine participate in this type of reaction.

Type II: Cytotoxic e.g., thrombocytopenia, hemolytic anemia, IgG, IgM and complement take part hi this reaction.

Type III: Immune complex or toxic complex e.g., Arthus reaction, serum sickness etc. In this reaction IgG, IgM and complement take part.

Type IV: It is delayed type of hypersensitivity in which T cells, lymphokines and macrophages take part e.g., tuberculin type and contact dermatitis.

Type V: It is antibody dependent cell mediate and cytotoxic type of reaction e.g., autoimmune orchitis in guinea pigs.

***Practical lesson* # 11**

Theme: **Immune serums and immunoglobulins. Reaction of flocullation (neutralization). Hypersensitivity. Autoimmune phenomena. Principles of the use of antibodies as medical, preventive and diagnostic preparations.**

Questions for the learning.

1. Assigning of immune serums, classification, application, reception.
2. Antitoxins, their characteristic. Antitoxic sera. Merits of E. Bering. Diagnostic serums, perception and application. Normal sera of the blood.
3. Gammaglobulines, structure, reception, application, mechanism of action.
4. Hypersensitivity. Types of hypersensitivity reactions and their mechanism of development.
5. Anaphylaxis. Serum sickness and its prevention.
6. Allergic reactions for immunodiagnosis of infectious diseases. Skin test (intradermal) and its diagnostic importanance.
7. Autoimmune phenomena.
8. Principles of the use of antibodies as medical, preventive and diagnostic preparations.

Independent work.

1. Fill in the table "Classification of immune serums"

№	Medical and prophylactic serums	Diagnostical serums
1.		
2.		
3.		

2. Give characteristics to the immunity, which occurs by using serums and immunoglobulins._____

_____

3. What is the immunoglobulin?

_____

_____

_____

4. Draw the structure of the *immunoglobulin molecule*.

5. What is the hypersensitivity?

_____

_____

5. Hypersensitivity reactions are classified into two main types, "immediate" and "delayed" types.

Distinguishing feature of immediate and delayed types of hypersensitivity.

Immediate hypersensitivity	Delayed hypersensitivity

6. **Coombs and Gell (1963) classified hypersensitivity reactions into 4 types based on the different mechanisms of pathogenesis**.

Types of hypersensitivity reactions and their features

Type of reaction	Clinical sindrome	Time required for manifestation	Mediators

7. Explain the term "autoimmune diseases" and fill in the table.

_____

_____

_____

Autoimmune diseases

Type	Diseases
1.	
2.	
3.	

Theme: **Vaccines. Principles of making and application of vaccines. Immunobiological preparations. Immune status of the human being. Estimation of the immune status. Immunomodulators. Immunocorrection. Transplantation immunology.**

Questions for the learning.

1. Artificial active immunity. Works of E. Jenner.
2. Scientific fundamentals of vaccines obtaining. Studies of L. Pasteur.
3. Classification of vaccines and requirements to them. Live vaccines, methods of obtaining. Inactivated (killed) vaccines. Ways of inactivating of vaccines strains. Chemical vaccines, method of obtaining. Anatoxins.
4. Concept about vaccination and revaccination.
9. Immune status of the human being**.** Estimation of the immune status.
10**.** Immunomodulators. Immunocorrection. Transplantation immunology.

Vaccines are immunological preparations using for establishing the active antiinfectious immunity by means of the mobilization of immune memory.

Adjuvants. The term "adjuvant" comes from the Latin word adjuvare, meaning "to help."

An adjuvant is a substance that, when added to a vaccine, greatly enhances its protection against infection.

A vaccine adjuvant is a substance that is added to the vaccine to increase the body's immune response to the vaccine.

There are many adjuvants, some of which are inorganic (such as alum), that also carry the potential to augment immunogenicity.

Two common aluminium salts include ***aluminium phosphate*** and ***aluminium hydroxide.***

These are the most common adjuvants in human vaccines.

Term	**Definition**
1	2
Immunological	Prevention of infectious diseases by creating immunity to

prophylaxis	them with immunological methods - active and passive immunization.
Immunotherapy	Treatment of patients by influencing the immune system.
Vaccines	Biological substances obtained from microorganisms, their metabolic products, synthetic, genetic engineering analogy, or anti-idiotype antibodies, that are used for active immunization of people for the prevention and treatment of infectious diseases.
Attenuation	Sustained irreversible weakening of the virulence of pathogenic microorganisms, it is used for obtaining vaccine strains.
Live vaccine	Consists of viable strains of pathogenic microorganisms with the most reduced virulence, but with safe antigenic properties. Live vaccine creates stress immunity, similar to postinfectious.
Inactivated vaccine	Inactivated vaccine (killed) consists of microorganisms that have expressed immunogenic properties, obtained under the action of physical and chemical factors.
Chemical vaccine	Consists of specific antigens that were extracted from bacteria and purified from ballast substances.
Toxoid	Qualitatively new medicine that is obtained from exotoxin by treatment with 0.3% solution of formalin at 37 ^{o}C for 30 days.
Genetic-engineering vaccine	It's obtained on the basis of microbe genomes sequence detecting: genes that control the required antigenic determinants, we transfer gene into other microorganisms and clone them, promoting the expression of these genes in the new environment.
Anti-idiotype antibodies	Vaccines, obtained from anti-idiotype antibodies that are characterized by similar structure between the antigen epitope and active center of anti-idiotype antibodies.

Antiserum	Serum, obtained from human or animals that were immunized with an antigen and contains some antibodies to this antigen, used for therapy or diagnostics.
Flocculation	A variety of precipitation reactions in the liquid in which the antigen-antibody complexes (often toxin-toxoid) form a visible precipitate (flocculants), which can be characterized quantitatively.
Toxoid activity	The smallest amount of toxoid which reacts in the flocculation reaction in the initial test-tube with one unit of antitoxic serum at 45 ^{o}C.
1	2
Initial tube	Initial tube – a tube, which contain equivalent amount of toxoid and antitoxin. Sediment appear for the first time in initial tube.

Indication for an assessment of immune status.

1. Tests of the first level of determination of immune status:

a – quantitative determination of T– and B–lymphocytes (E and EAC rosette–formation test);

b – determination of the concentration of the main classes of immunoglobulins;

c – determination of phagocytic activity of leukocytes.

2. Tests of the second level (analytical) for assessment of immune status:

a – determination of subpopulations of T lymphocytes;

b – the macrophage migration inhibition test;

c – cutaneous tests of hypersensitivity;

d – examination of proliferative ability of T– and B–lymphocytes (lymphocyte blast transformation test);

e – assessment of activity of K–cells and NK–cells;

f –examination of the components of the complement system;

g – assessment of different stages of phagocytosis.

3. Immunedeficiencies.

4.1. Primary immunodeficiency:

a – B–cell deficiencies;

b – T-cell deficiencies;

c – combined immunodeficiency;

d – complement deficiency and phagocytosis disturbances.

4.2. Secondary immunodeficiency.

5. Autoimmunity.

7. Tumour immunology.

8. The methods of immune correction. Immunomodulators.

Indication for an assessment of immune status.

1. Detailed examination of the human health.
2. Genetic defects of the immune system (primary immunodeficiency).
3. Acute and chronic bacterial, viral and protozoan disease (hepatitis, sepsis, chronic pneumonia, leishmaniasis, AIDS etc.).
4. Autoimmune diseases (rheumatism, rheumatoid arthritis, systemic lupus erythematous, etc).
5. Dermatoveneral diseases (contact dermatitis, pemphigus, mycosis fungoides, syphilis, etc.).
6. Tuberculosis and leprosy.
7. Allergic diseases (bronchial asthma, atopy, etc.).
8. Primary diseases (multiple sclerosis, etc.).
9. Malignant tumours (leukosis, lymphogranulomatosis, lymphosarcoma etc.).
10. Normal graviditas, pathological pregnancy (toxicosis, Rh-incompatibility, repeated abortions, etc.).
11. Psychical diseases (narcomania, schizophrenia, etc.).
12. Starvation.

13. Examination of the patients in gerontological and endocrinological hospitals.
14. The control of cytostatic , immunosuppressive and immunostimulation therapy.
15. Examination of the recipients before and after transplantations.
16. Evaluation of immune system state in patients before difficult planed operations.
17. Scientific and practical examinations (studying of new types of action, physiotherapy, influences of new types of narcosis and new types of drugs, etc.).
18. During prophylactic medical examination (the tests of the first level).

The first level tests for assessment of immune status (approximate):

1. Determination of total quantity of lymphocytes in periferal blood (absolute and relative);
2. Determination of T– and B–lymphocytes in peripheral blood;
3. Determination of the concentration of the main classes of immunoglobulins;
4. Determination of phagocitic activity of leukocytes.

The second level tests for assessment of immune status (analytical):

1. Determination of subpopulations of T lymphocytes ($CD4^+$ and $CD8^+$);
2. Leukocyte migration inhibition test;
3. Examination of proliferative ability of T– and B–lymphocytes (lymphocyte blast transformation test);
4. Determination of specific IgE;
5. Cutaneous tests of hypersensitivity;
6. Determination of circulating immune complexes;
7. Determination of B-lymphocytes which carry superficial immunoglobulins of different classes;
8. Assessment of immunoglobulins synthesis in B-lymphocytes culture;
9. Assessment of activity of K–cells and NK–cells;
10. Examination of the components of the complement system;
11. Assessment of different stages of phagocytosis;
12. Assessment of different mediators and interleukin-producing activity of the cells.

Some indexes of human immune state

Indexes	Norm
Absolutely number of leukocytes (10^9/L)	4-8
Absolutely number of lymphocytes (10^9/L)	0.8-3.6
Lymphocytes (%)	18-38
Neutrophils (%)	50-77
Phagocytic index (%)	50-70
Phagocytic cells number	3-9
Bactericidal activity of blood serum (%)	50 %
Complement titre	0.02-0.08
IgA (g/L)	1.4-2.0
IgG (g/L)	0.8-1.5
IgM (g/L)	8.0-12.0
IgE (g/L)	0.0002
T-lymphocytes in E-RFT ((10^9/L)	0.6-1.6
T-lymphocytes	40-60
B-lymphocytes in EAC-RFT (10^9/L)	0.2-0.4
B-lymphocytes (%)	15-30
NK cells (%)	5-20
Th cells (10^9/L)	0.3-0.7
Th cells (%)	30-40
Ts cells (10^9/L)	0.2-0.4
Ts cells (%)	15-20
Th/Ts	1.2-3.0
Heteroagglutinins titre	2.5-3.0

Indexes	Norm
Circulating immune complexes	0.2
Lymphocytes blast transformation test with phytogemagglutinin (%)	50-75

Practical lesson # 12

Theme: **Vaccines. Immunopathology. Estimation of the immune status.**

<u>Questions for the learning.</u>

1. Artificial active immunity. Works of E. Jenner.
2. Scientific fundamentals of vaccines obtaining. Studies of L. Pasteur.
3. Classification of vaccines and requirements to them.
4. Live vaccines, methods of obtaining.
5. Inactivated (killed) vaccines. Ways of inactivating of vaccines strains.
6. Chemical vaccines, method of obtaining.
7. Anatoxins.
8. Concept about vaccination and revaccination.
9. Immunodeficiency. Autoimmune diseases. Immune status of the human being**.**

Independent work.

1. Explain the term "vaccine".

_____

_____

_____

2. Fill in the table.

Classification of vaccines

Types of the vaccines	Examples of the vaccines
1.	

2.	
3.	
4.	
5.	

3. Give characteristics of the immunity, which occurs by using vaccines.

_____
_____
_____

4. What is the adjuvants? Name several examples.

_____
_____
_____

5. Explain the term "immunodeficiency disorders" and name its classification.

_____
_____
_____
_____
_____
_____
_____

6. Explain the term "autoimmune diseases" and fill in the table.

Autoimmune diseases

Type	Diseases
1.	
2.	
3.	

7. Explain the term "immune status of the human being" and methods of its studying.

_____
_____
_____
_____

8. Fill in the table " Immune status of the human being"

Immune parameter	Normal

Practical lesson # 13

Submodule 2. **Infection and immunity.**

1. The doctrine of immunity. Stages of development of immunology. Types of immunity and its forms of manifestation.

2. Nonspecific factors of the organism protect from pathogenic microbes. Complement, its properties, ways of activation. Cytokines. Phagocytosis, phagocytic cell types. Stages of phagocytosis. Completed and incomplete phagocytosis.

3. The immune system of the organism and its organs. The role of the thymus in the immune response. Cells of the immune system, their types, the interaction of T-, B-lymphocytes and macrophages. Their role in the cellular and humoral immunity.

4. Regularities of immune response. Phases of the immune response. Immunological reactions. Immunological tolerance, reasons for its occurrence. Immunological memory and its mechanism.

5. Immediate and delayed hypersensitivity types, their mechanisms, the differences. Practical value.

6. Three cells cooperation system of the immune response. The role individual immune system cells and their interaction. Interleukins.

7. Antigens and their characteristics. Full and defective antigens. Antigenic structure of bacteria. Practical value of the study about microbial antigens. Autoantigen. Antibodies, their nature. Place of synthesis, the dynamics of production of antibodies. Autoantibodies.

8. Antitoxins, their properties, mechanism of action. Principles of receiving of antitoxic serums. Units of measurement, practical use.

9. Serological reactions, their characteristics, main types, practical use. Agglutination reaction, its mechanism and types. The practical use.

10. Precipitation reaction and its mechanism. Use in medical practice. Gel precipitation test.

Serological tests. Reactions of lysis. Complement fixation test, its practical use.

11. Reactions with labeled antibodies or antigens. Practical use of immunofluorescence reaction (IFR), ELISA and radioimmune assay.

12. Forms and types of immune response. Humoral immune response and its stages. Primary and secondary immune response.

Interaction of immune system cells in the immune response process.

13. Reactions of the immune response, their characteristics. Cellular immune response.Immediate and delayed hypersensitivity types. The mechanism of these reactions.

14. Monoclonal antibodies, their production and use in medical practice.

15. Immunodeficiency conditions, autoimmune processes. Complex evaluation of the immune status of the organism.

16. Live vaccines, the principles of their production. Control, the practical use of live vaccines, evaluation of effectiveness.Vaccines. History of receipt.

17. Classification of vaccines. Corpuscular, chemical, synthetic, genetically engineered and antiidiotypic vaccines.

18. Chemical vaccines and toxoids, principles of receipt. Associated vaccine. Adsorbed vaccine, the principle of "repository".

19. Toxoids, their reception, cleaning, measurement units, evaluation and the practical use.

20. Corpuscular vaccine from killed microbes. Principles of receipt, control of, evaluation of effectiveness.

Recommended reading list

Main iterature

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2. Gaidash I. Microbiology, Virology and Immunology. Vol. 1 / I. Gaidash, V. Flegontova; Ed. N. K. Kasimirko. - Lugansk : S. N., 2004. - 213 p.
3. Gaidash I. Microbiology, Virology and Immunology. Vol. 2 / I. Gaidash, V. Flegontova; Ed. N. K. Kasimirko. - Lugansk : S.N., 2004. - 226 p.
4. Jawetz, Melnik & Adelberg's Medical Microbiology [Текст] : учебное пособие. - 22 Edition. - New York : Lange Medical Books/McGraw-Hill, 2001. - 695 p.
5. Medical Microbiology : textbook / D. Greenwood [et al.]. - 17th ed. - Toronto : Churchill Livingstone, 2007. - 738 p.

Further Reading

1. Talaro K. Faundations in microbiology. Basic principles. - Talaro K., Talaro A. - Pasadena, 2005, by TMHE group.
2. Microbiology. A human perspective / M. T. Nester, E. V. Nester, C. E.Roberts. - 1995.
3. Levenson W. E. Medical microbiology and immunology / W. E. Levenson, E. Javetz. – Norwalk, 1994,
4. Krivoshein Yu. S. Handbook on microbiology / Yu. S. Krivoshein– Moscow : Mir Publishers,.1989

Informational resourses:

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2. http://*www.yteach.co.uk/in*dex.php/search/results/AQA_GCSE Science_A(4461)_Biology,3,0,7033;7230,0,25,1,wa,1.html
3. American Society for Microbiology — http:// asm.org.;
4. http://journals.asm.org; (American Society for Microbiology) — http:// asm.org.;
5. http://www.news-medical.net/health/Virus-Microbiology-(Russian).aspx;
6. http://www.rusmedserv.com/microbiology; http://www. rusmedserv.com/
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8. http://www. infections.ru/rus/all/mvb journals.shtml;
9. http://dronel.genebee.msu.su/journals/ microb-r.html.