MINISTRY OF HEALTH SERVISE OF UKRAIN

ZAPOROZHYE STATE MEDICAL UNIVERSITY

THE CHAIR OF MICROBIOLOGY, VIROLOGY AND IMMUNOLOGY

Immunity.
Factors and mechanisms of the immunity.

Practicum on Microbiology, Virology and Immunology

for foreign students
of II-III courses of the medical faculty,
specialty "Medicine"

Zaporozhye – 2019

UDC: 578.28.083.3(075.8) = 111

Y 45

Ratified on meeting of the Central methodical committee
of Zaporizhzhia State Medical University
(protocol N_____from_____)
And it is recommended for the use in education process for foreign students.

AUTHORS:

Yeryomina A. K., senior lecturer of the chair of microbiology, virology and immunology, candidate of Biological Sciences**.**

Kamyshny A. M., the heat of the chair of microbiology, virology, and immunology, doctor of medicine, professor.

Sukhomlinova I. E., assistant professor of the chair of normal physiology**,** candidate of Medicine.

REVIEWERS:

Popovich A.P., docent of the Chair of Medical Biology, candidate of Medicine.

Tykhonovska M.A., docent of the Chair of normal physiology**,** candidate of Medicine.

Yeryomina A. K.

Y 45 Immunity. Factors and mechanisns of the immunity : practicum on microbiology, virology and immunology for the students of II- III course of the medical faculty/ Yeryomina A. K. [et al.]. – Zaporizhzhia, 2019. – 87 p.

UDC: 578.28.083.3(075.8) = 111

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ

Запорізький державний медичний університет

Кафедра мікробіології, вірусології та імунології

Імунітет. Фактори та механізми імунітету.

Практикум з мікробіології, вірусології та імунології

для іноземних студентів
ІІ-ІІІ курсів медичного факультету,
спеціальність «Медицина»

Запоріжжя - 2019

Практикум з мікробіології, вірусології та імунології для іноземних студентів II-III курсів медичного факультету, спеціальність «Медицина».

Автори:
старший викладач кафедри , к.біол.н. **Єрьоміна А.К.,**
зав. кафедри, д.мед.н., професор **Камишний О.М.,**
доцент кафедри нормальної фізіології, к.мед.н. **Сухомлінова І.Є.**

Рецензенти:

доцент кафедри нормальної фізіології **Тихоновська М.**
доцент кафедри медичної біології, паразитології та генетики **Попович А.П.**

Затверджено ЦМР ЗДМУ: протокол № 5 від 23.05. 2019р.

IMMUNITY.

FACTORS AND MECHANISMS OF THE INNATE IMMUNITY

Theme topicality. Immune system is the most important for sustainability of internal environment (homeostasis) of human body. There is not any inflammatory pathological process, which is not linked with the state of immunity. The termination of the pathological process depends primarily on the state of immunity.

Different specialists (infectious diseases, therapuetics, surgery, oncology, etc.) need the knowledge of the immunology for understanding the essence of illness, timely diagnosis, treatment, and application of diseases and complications prevention.

This theme contains information about congenital factors and mechanisms of the immunity.

Primary objective: to be able to estimate the status of cellular and humoral factors of innate immunity.

QESTIONS FOR DISCUSSION

1. Definition of immunity. Role of immunity factors and reactions in the infectious and noninfectious acquired human pathology.
2. Innate and acquired factors of the immunity. The first line of the protection.
3. Types of the anti-infectious immunity.
4. Humoral factors and mechanisms of innate resistance (IR), their definition and function.
5. Cellular factors and mechanisms of IR. Their definition and function. Value of Mechnikov's works.
6. The role of immunology in the development of medicine. Immunodiagnosis, immunotherapy, and immunization.

PROCEDURE OF PRACTICAL SESSION

Study phagocytosis of the latex by staphylococcus. Stain the smears by methylene blue.

For investigation we isolate the phagocytes from blood with using standart procedure. Latex with concentration 100 partical on 1 leucocyte is added to 0,2 ml of the phagocytes. Tube with components is sheked and incubated at 37 °C with 90

minutes. The smears are making from suspension after incubation, and fixing by Nikiforov liquid, stain it by Romanovsky-Gimse method. Find in the smear leucocytes, which are phagocyted latex particles. Calculate 100 neutrophyles in the smears. Phagocytic numerous is amount of the cells that phagocytes the latex particles. Phagocytic index is media numerous of the latex particles in a phagocyte cell. Draw them.

Define phagocytosis activity in the preparation (the count of phagocytic activity and phagocytic index).

Determination of phagocytic activity (FA) and phagocytic index (FI) is carried out in the preparation. Take 0.2 ml of the blood in a child with staphylococcal pneumonia and mix it with sodium citrate solution for the smears preparation. Then add 0.12 ml of 2 billion staphylococcus suspension. After 30 minutes incubation in a thermostat with blood, smears are to be prepared and stained by Romanovsky's method to examine phagocytosis indices. In different fields of view count at least 50 white blood cells and total number of microbes in them. Identify the percentage of phagocytes, which have taken at least one staphylococcus cell (FA) and the average number of microbes that are absorbed by cell (FI). Write a conclusion.

Study the demonstration and record it in the protocol.
Determination of lysozyme activity in saliva.

Lysozyme is a proteolytic muramidase enzyme (lat. *murus* – wall). Hydrolysis of acetylamino-polysaccharides of bacterial cells lead to the disturbance of cell wall synthesis. Lysozyme is mucosal protective factor and it is present in tears, saliva, blood, and mother's milk.

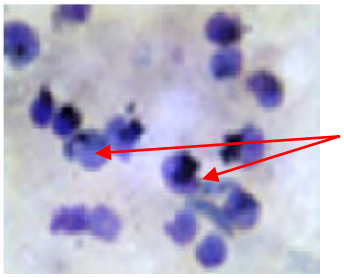
For determination of the lysozyme titre in saliva make a series of the consecutive dilutions of saliva (1:10, 1:100, 1:1000, 1:10000). The last tube is a control one and it does not contain lysozyme. 1 ml *Micrococcus lysodeicticus* suspension contain 1 billion microbial cells is added in all five tubes. After incubation in a thermostat for 3 hours titre of lysozyme is definite. It is the last dilution in which lysis of bacteria occurs.

Determination of bactericidal activity in the blood serum. Germicidal activity of blood serum is the ability to eliminate pathogenic bacteria. This ability is caused by the influence of non-resistance humoral factors (lysozyme, lactoferine, complement) and normal antibodies.

Experiment 1. Prepare a series of serum dilution, and then add in every tube a standard suspension of bacteria.

Experiment 2. After incubation in a thermostat make the inoculation of each dilution on the medium. The largest dilution of the serum in which there is no growth of bacteria is a titre of the bactericidal activity of the blood.

Definition of the bactericidal action of the neutrophils with the help of the nitro blue tetrazolium recovery test (NBT-test).



NBT test

The principle of the test is restoration of the soluble colourless nitro blue tetrazolium in diformazan, which is distributed in the cytoplasm or on the surface of phagocytes in the form of granules, coloured in dark blue, when neutrophils are activated. This test reflects the degree of oxygen-depending mechanisms activation of the phagocyte cells bactericidal activity, the basis of which is activation of NADF-H_2-oxydase and hexomonophosphate shunt. In the result of a series of reactions, active oxygen is generated (superoxide anion O_2-). It has bactericidal activity and renews nitro blue tetrazolium.

Immunity. Factors and mechanisms of the innate immunity

Notion	Definition/explanation
Immunity	Dynamic condition of the organism that consists of the set of specific

Notion	Definition/explanation
	and nonspecific, humoral, and cellular factors and responses, which provides stability (constancy) of internal environment (homeostasis) of the organism
Types of immunity	1. Innate. 2. Acquired
Types of acquired	1. Natural (active and passive).

immunity	2. Artificial (active and passive)
Mechanisms of immunity	1. Cellular. 2. Humoral. 3. Pathophysiological
Nonspecific mechanisms of cellular immunity	1. Bactericidal activity of the skin and mucous membranes. 2. Barrier capacity of lymph nodes. 3. Phagocytosis. 4. Functions of natural killer. 5. Inflammation. 6. Cell areactivity
Toll-like receptors	Toll-like receptors (TLRs) are surface receptors that allow cells to "see" molecules that signify the presence of microbes outside of the cell; NOD proteins do the same for the inside of a cell (the cell's cytoplasm).
Cellular factors of the innate immunity	Phagocytes (macrophages, monocytes, microphages - neutrofils), natural killer cells.
Phagocytes	Cells that routinely engulf and digest material, including invading organisms.
Phagocytosis (types)	Phagocytosis involves a series of complex steps. These are particularly important medically, because most pathogens have evolved the ability to evade one of them. The steps of phagocytosis include: **1. Chemotaxis.** The phagocytic cells are recruited to the site of infection or tissue damage by certain chemical stimuli that act as chemoattractants. These include products of microorganisms, phospholipids released by injured mammalian cells, and the complement component C5a. **2. Recognition and attachment.** Phagocytic cells use various receptors to bind invading microbes either directly or indirectly. Direct binding occurs through receptors that recognize patterns associated with compounds found on microbes. For example, one type of receptor on phagocytic cells binds mannose, a sugar found on the surface of some bacteria and yeasts. Indirect binding occurs when a particle has first been opsonized, dramatically enhancing the phagocytes' ability to attach and subsequently engulf the material. Opsonins include the complement component C3b and certain classes of antibody molecules; phagocytes have receptors for specific parts of these molecules. **3. Engulfment.** The phagocytic cell engulfs the invader, forming a membrane-bound vacuole called a phagosome. This process involves rearrangement of the phagocyte's cytoskeleton, forming armlike extensions called pseudopods that surround the material being engulfed. Engulfment itself does not destroy the microbe. **4. Fusion of the phagosome with the lysosome.** Within the phagocyte, the phagosome is transported along the cytoskeleton to a point where it can fuse with lysosomes, membranebound bodies filled with various digestive enzymes, including lysozyme and proteases. The fusion results

Table continuation

Notion	**Definition/explanation**
	in the formation of a phagolysosome **5. Destruction and digestion.** Within the phagolysosome, oxygen consumption increases enormously as sugars are metabolized via aerobic respiration, with the production of highly toxic oxygen products such as superoxide, hydrogen peroxide, singlet oxygen, and hydroxyl radicals. As the available oxygen in the phagolysosome is consumed, the metabolic pathway switches to fermentation with the production of lactic acid, lowering the pH.

	Various enzymes degrade peptidoglycan and other components of the bacterial cell. **6. Exocytosis.** Following digestion of the microorganisms, the membrane-bound vesicle fuses with the plasma membrane, expelling the digested material to the external environment
Phagocytosis (types)	Complete Incomplete Immune
Phagocytosis estimation indices	- restoration of the nitro blue tetrazolium test; - chemotaxis capacity; - phagocytic index (FI); - phagocytic numerous (FN)
Natural killers (NK)	Large granular lymphocytes, which do not have markers of T and B lymphocytes. They make rapid cytolysis of foreign cells based on lectin recognizing. It is an important factor in antitumor and antiviral protection
Humoral factors of the innate immunity	1. Complement system (C1 - C9). 2. Interferon system (α, β, γ). 3. Cytokine produced by monocytes and macrophages (IL-1, TFN α, IL-6, chemokins). 4. Acute phase proteins. 5. Eucosanoids. 6. Properdin. 7. Lysozyme
The complement system	The complement system is a series of proteins that constantly circulate in the blood and the fluid that bathes the tissues. Early studies showed that these proteins augment the activities of the adaptive immune response; in fact, their name is derived from observations that they "complement" the activities of antibodies. They routinely circulate in an inactive form, but in response to certain stimuli indicating the presence of foreign material, a cascade of reactions occurs. This results in the rapid activation of critical complement system components. These activated forms have specialized functions that cooperate with other host defences to quickly remove and destroy the offending material. Three pathways lead to the activation of the complement system: Classical pathway - initiated by antigen-antibody complexes; Alternative pathway - initiated by binding of C3b to cell surfaces (regulatory proteins protect host cell surfaces); Lectin pathway - binding of mannan-binding lectins to cell surfaces
Outcomes of the complement system activation	**Inflammation.** The complement components C3a and C5a induce changes in endothelial cells that line the blood vessels, and in mast cells. These effects contribute to the vascular permeability associated with inflammation. C5a is also a potent chemoattractant, drawing phagocytes into the area where complement was activated

Notion	Definition/explanation
	Lysis of foreign cells. Complexes of C5b, C6, C7, C8, and multiple C9 molecules spontaneously assemble in the membranes of cells, forming doughnut-shaped structures each called a membrane attack complex (MAC). This creates pores in the membrane, disrupting the integrity of the cell. Note that the membrane attack complex has little effect on Gram-positive bacteria because their peptidoglycan layer prevents the complement components from

	reaching their cytoplasmic membrane. The outer membrane of Gram-negative bacteria, however, renders them susceptible **Opsonization.** The complement protein C3b binds to foreign material. Phagocytes more easily "grab" particles coated with C3b because phagocytic cells have receptors for the molecule on their surface. The material that C3b has coated is said to be opsonized (which means "prepared for eating"); compounds such as C3b that can opsonize material are opsonins. Opsonized material may be viewed as carrying a giant "eat me" sign that can be read by phagocytes. Our own cells are protected from the effects of C3b because our membranes bind regulatory molecules, leading to the inactivation of C3b when it binds
Acute phase proteins (APP)	1. C-reactive protein. 2. Fibrinogen. 3. Mannose-connective protein. 4. Serum amyloidal protein. Function of APP is associated with inflammation and elimination of the pathogens from the body
Cytokine	Mediators of intercellular communication at the immune response, haemogenesis, inflammation. They are based on the receptor mechanism. Cytokines include interleukin, chemokin, interferon, tumor necrosis factor and granulocyte colony stimulating factor (G-CSF). They are determined with diagnostic purpose and are used as immunotherapeutic agents
Eucosanoids	Active products of arachidonic acid metabolism (leucotrienes, prostaglandins)
Acquired factors of the adaptive immunity	Antibodies against specific antigens of causative agents during humoral immune responses. There are T killers (cytotoxic), sensitized delay T lymphocytes, and activated macrophages during cell immune responses
The first line of protection	Humoral and cellular factors that determine the protection of the organism from pathogens to the formation of acquired humoral and cellular immunity factors
Immunodiagnosis	System of the laboratory methods that are based on immune reactions and used for diagnosis. These reactions determine the molecules of antibodies or antigens (serodiagnosis, the definition of antigenic properties of the microorganisms), making the skin allergic tests (determination-sensitized lymphocytes)
Immunotherapy	Methods of infectious and noninfectious diseases treatment, that are aimed at the antigens binding and normalization of immune system disorders using immunological agents (immune sera, immunoglobulin and other immunostimulants or immunosuppressants)
Immunization	A system of specific and nonspecific immunological factors, that are aimed in most cases at stimulation of immune responses by vaccines and other drugs for prevention of the diseases

ANTIGENS

Theme topicality. Antigens have the leading role in the development of immune responses and in infectious process. Various antigens of the microorganisms are used in medical practice for diagnostics, prevention, and sometimes treatment of the infectious diseases.

Primary objective: to be able to distinguish different antigens and to use them in medical practice.

QESTIONS FOR DISCUSSION

1. Antigens: identification, structure (epitopes, carriers).
2. Classification of the antigens by origin, chemical nature, level of the immunogenicity.
3. The main properties of the antigens: antigenicity, immunogenicity, specificity.
4. Antigens of the human body: blood group antigens, self-antigen. Major histocompatibility complex antigens: identification, localization, HLA system, nomenclature, functions, role in the immune response.
5. CD–antigens of the immune system cell.
6. Antigens of the microorganisms (bacteria, viruses) and pathogenicity.
7. Antigen processing in the body. Superantigens.
8. Practical use of the microorganisms antigens.

PROCEDURE OF PRACTICAL SESSION

Study and record in the protocol demonstration antigenic preparations:

1. Preparations used for diagnosis: antigens, diagnosticums, and erythrocytic diagnosticums.
2. Preparations used for internal skin-allergic tests for diagnostic purposes– allergens.
3. Preparations used for preventive purposes–vaccines.

It is necessary to write antigenic preparations by groups according to their purpose of use in practice. Record the name of each drug, their characteristics, and use. Draw a scheme of the toxoid preparation.

Diagnostic preparations:

1. Diagnosticums and antigens:

- brucelosis diagnosticum;
- typhoid, paratyphoid O and H diagnosticum;
- polysaccharide antigen of *Candida albicans*;
- salmonellosis diagnosticum;

- dysenteria diagnosticum *Sonnei* and *Flexneri*;
- typhoid fever erythrocytic diagnosticum;
- whooping cough erythrocytic diagnosticum;
- gonococcal antigen;
- whooping cough antigen;
- salmonellosis erythrocytic O diagnosticum.

2. Allergens for skin-allergic test

- tuberculin;
- brucellin;
- antraxin;
- tularin;
- allergens of *Candida* fungi;
- trichophytin.

Preparation for treatment and prevention:

1. Vaccines

1)live (attenuated) vaccines against: tuberculosis (BCG), polio, measles; mumps, rubella, brucellosis, influenza, anthrax, plague, tularemia, yellow fever, Qu-fever, smallpox.

2) killed (inactivated) vaccine against: pertussis, gonorrhea, influenza, rabies, tick-borne encephalitis, autovaccine, cholera, leprosy, leptospirosis, brucellosis.

3) toxoids against: diphtheria, tetanus, staphylococcus, cholera, botulism (A, B, C, D, E), gas anaerobic infection.

4) c*hemical vaccine against:* typhoid fever, meningococcal infection, influenza, haemophilic infection.

5) *recombinant (genetically engineering) vaccine against:* hepatitis B.

6) associated vaccines against: APDT (adsorbed whooping-diphtheria-tetanus);
sexta toxoid (against botulism types A, B, E, tetanus, *Cl. perfringens, Cl. novyi).*

2. Vaccines used for treatment:

1) killed (inactivated) against:

- brucellosis;
- gonorrhoea;

auto-vaccine at chronic staphylococcal infection;

2) *toxoid against:*

- antistaphylococcu

Antigens

Notion	Definition/explanation
Antigens	Foreign macromolecules that when introducted into the body cause the formation of the immune response, on condition of their recognition by specific receptors of lymphocytes
Structure of the antigen molecule	Molecule consists of the antigen epitopes that are located on the surface of the molecule and determine the specificity of their carrier, which is often represented by protein molecule. Different chemical groups often represent epitopes: NH2, COOH, NH-OOH, etc.
Conditions of antigenicity	Foreignness, macromolecularity (molecular weight is not less that 20-30 kD, colloidal state, catabolism processing capacity in the macrophages and other cells
Classification of antigens	Exogenous, endogenous, protein, polysaccharide, glycoproteins, nucleoproteins, viral, macroorganism cells antigens, complete, incomplete – haptens, self-antigens, allergens
Bacteria antigens	H (flagella), O (somatic), K (capsular)
Structures concerning antigens	Different structures of bacteria, viruses, toxins, blood products of blood serum, blood group antigens, organs and tissues that are transplanted to another organism – recipient
Allergens	The varieties of antigens that cause allergic reaction
The main features of antigens	**Immunogenicity** is the ability to induce immune response. Immunogenicity depends on the molecular weight, structure of the antigen molecules, and genotype of the responsdent body. **Antigenicity** is the ability to react (bind) with specific antigens. **Specificity** in the immune organism is the ability to react only with those antibodies and effector cells, which were formed under the influence of the antigen in the body. **Specificity** in nonimmune body (the first entering) is the ability to react only with those clones of lymphocytes that have receptors for this antigen
Autoantigens	Altered self-antigens in cells and tissues of the body changed under the influence of intracellular organisms, viruses, temperature, and other factors. They are cells of specialized organs (thyroid gland, brain cells, sex gland, etc.)
Species and type specific antigens	Antigens that concern only one species or the type of the microorganisms and tissues

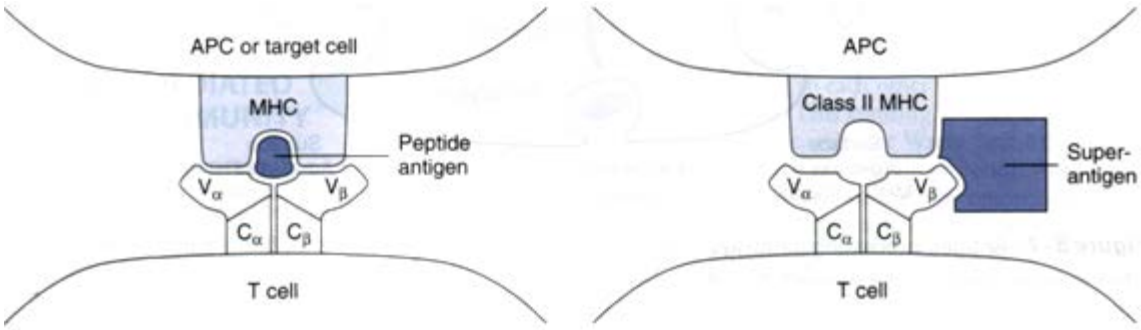
Table continuation

Notion	Definition/explanation
Functions of the antigens	The functions of the antigens are inducing of the immune response in the body and interact with specific antibodies and effectors cells, which are formed under the influence of the antigen
Haptens	Imperfect antigens, which have no immunogenicity, but have a specificity and can react with appropriate antibodies and cells. Haptens are stains, chemical preparations, antibiotics, etc.
Practical use of microorganisms antigens	It is based on their specificity. They are used for serological diagnosis of the infectious diseases. Antigens are used for detection the specific antibodies in serum of the patients. Allergens are used for diagnosis of the diseases (tuberculosis, brucellosis, anthrax, etc.) in skin-allergic test. Allergens are introduced intra-cutaneously. Such bacteria

	antigens as exotoxins are used to create different vaccines for preventive purposes
Antigen processing and presentation	Antigen processing and presentation are the means by which antigens become associated with molecules of the MHC for presentation to T cells with appropriate receptors. Proteins from exogenous antigens, such as bacteria, are internalized via endocytic vesicles into antigen-presenting cells such as macrophages. Then they are exposed to cellular proteases in intracellular vesicles. Peptides, approximately 10 to 30 amino acid residues in length, are generated in endosomal vesicles. The endosomal vesicles can then fuse with exocytic vesicles containing class II MHC molecules. The class II MHC molecules are synthesized, as for other membrane glycoproteins, in the rough endoplasmic reticulum and then proceed out through the Golgi apparatus. A third polypeptide, the invariant chain (Ii), protects the binding site of the class II (αβ) dimer until the lowered pH of the compartment created after fusion with an endosomal vesicle causes a dissociation of the Ii chain. The MHC class II-peptide antigen complex is then transported to the cell surface for display and recognition by a T cell receptor of a CD4 T cell. Endogenous antigens are cytosolic viral proteins synthesized in an infected cell and processed for presentation by class I MHC molecules. In brief, cytosolic proteins are broken down by a peptidase complex known as the proteasome. The cytosolic peptides gain access to nascent MHC class I molecules in the rough endoplasmic reticulum via peptide transporter systems (transporters associated with antigen processing; TAPs). The TAP genes are also encoded in the MHC. Within the lumen of the endoplasmic reticulum, peptide antigens complex with nascent MHC class I proteins and cooperate with β2 microglobulin to create a stable, fully folded MHC class I-peptide antigen complex that is then transported to the cell surface for display and recognition by CD8 cytotoxic T cell. The binding groove of the class I molecule is more constrained than that of the class II molecule, and for that reason shorter peptides are found in class I than in class II MHC molecules. Several viruses attempt to affect the immune response by interfering with the antigen-processing pathways

Notion	**Definition/explanation**
	10% of T cells to be nonspecifically activated. Examples of superantigens include certain bacterial toxins, including the staphylococcal enterotoxins, toxic shock syndrome toxin, and group A streptococcal pyrogenic exotoxin A. These antigens bind to the "outside" of the MHC protein and to the T cell receptor. They are active at very low concentrations ($10^{\sim 9}$ mol/L) and cause T cells expressing particular Vβ sequences to be stimulated and to release large amounts of cytokines, including IL-1 and tumor necrosis factor (TNF). It is the release of large amounts of cytokines from stimulation of a high percentage of the pool of T lymphocytes. They explain to large extent the pathogenesis of diseases caused by organisms expressing superantigen
CD antigens	T-cell receptors are associated with their so-called co-receptors, other membrane-enclosed proteins are expressed on the T cell surface which include the multiple-chain CD3 complex, and CD4 or CD8 molecules (depending on the specific differentiation of the T cell). CD

	stands for “cluster of differentiation” or “cluster determinant” and represents differentiation antigens defined by clusters of monoclonal antibodies
The main histocompatibility complex (MHC)	It is the genetic locus in the sixth human chromosome. It contains three groups of genes I, II, and III classes. Derivative of these genes is the system of HLA (human leukocyte antigens). Genes of I and II classes are characterized by extremely high polymorphism (they have several tens allele forms). This cause their function in the tissue incompatibility. Biological function of the gene products MHC of I and II classes is the presentation of the antigenic peptides to the T-lymphocytes, respectively II CD4 and CD8. II class of genes determines the level of the immune response



Binding of antigen by MHC and T cell receptor

Antigens

Antigen is foreign macromolecule

INFECTIOUS

BACTEIAL

Corpuscular (whole cells)

Extracellular: exotoxins, pathogenic enzyme (DNAse, coagulase, lecitinase, hyaluronidase)

VIRUS: Proteins, glycoproteins, lipoproteins, enzymes (neurominidase, haemagglutinin)

SOLUBLE: Proteins, polysaccharides, lipoproteins, endotoxins, exotoxins

H-Ag (flagellar), O-Ag (somatic), K-Ag (capsular)

NONINFECTIOUS

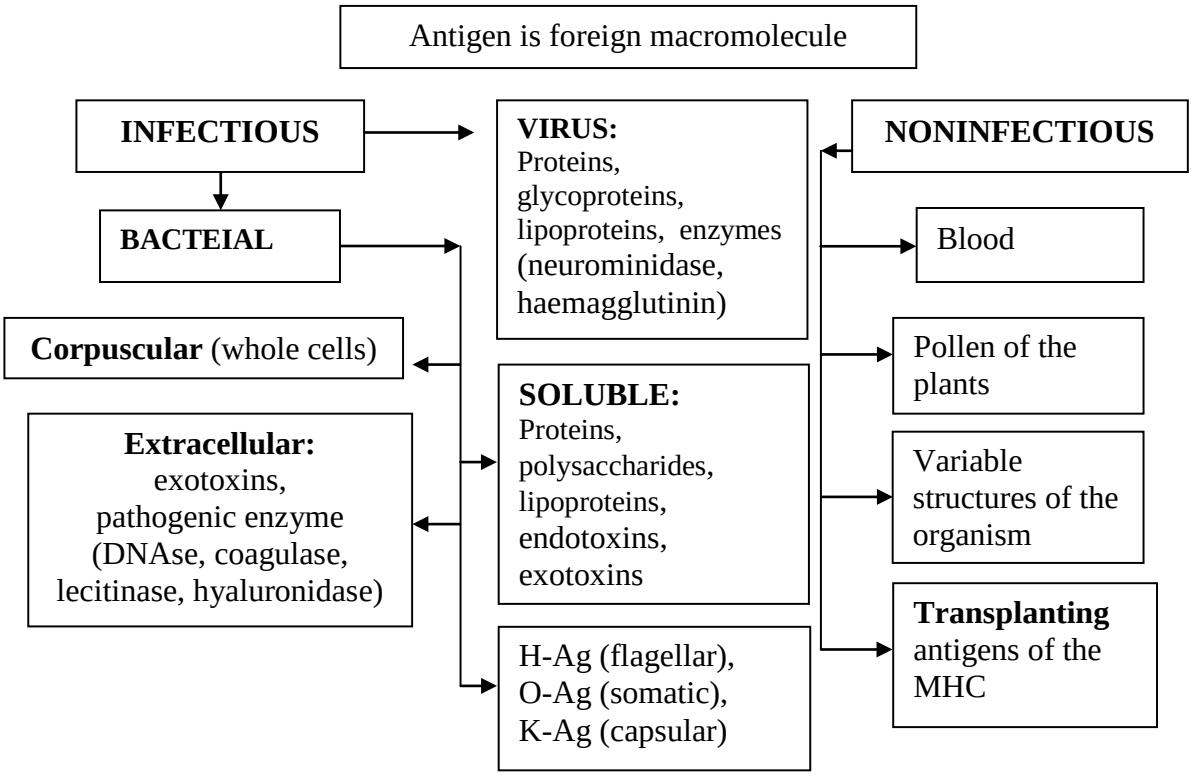
Blood

Pollen of the plants

Variable structures of the organism

Transplanting antigens of the MHC

Antigens


Antigen is foreign macromolecule
INFECTIOUS
BACTEIAL
Corpuscular (whole cells)
Extracellular: exotoxins, pathogenic enzyme (DNAse, coagulase, lecitinase, hyaluronidase)
VIRUS: Proteins, glycoproteins, lipoproteins, enzymes (neurominidase, haemagglutinin)
SOLUBLE: Proteins, polysaccharides, lipoproteins, endotoxins, exotoxins
H-Ag (flagellar), O-Ag (somatic), K-Ag (capsular)
NONINFECTIOUS
Blood
Pollen of the plants
Variable structures of the organism
Transplanting antigens of the MHC

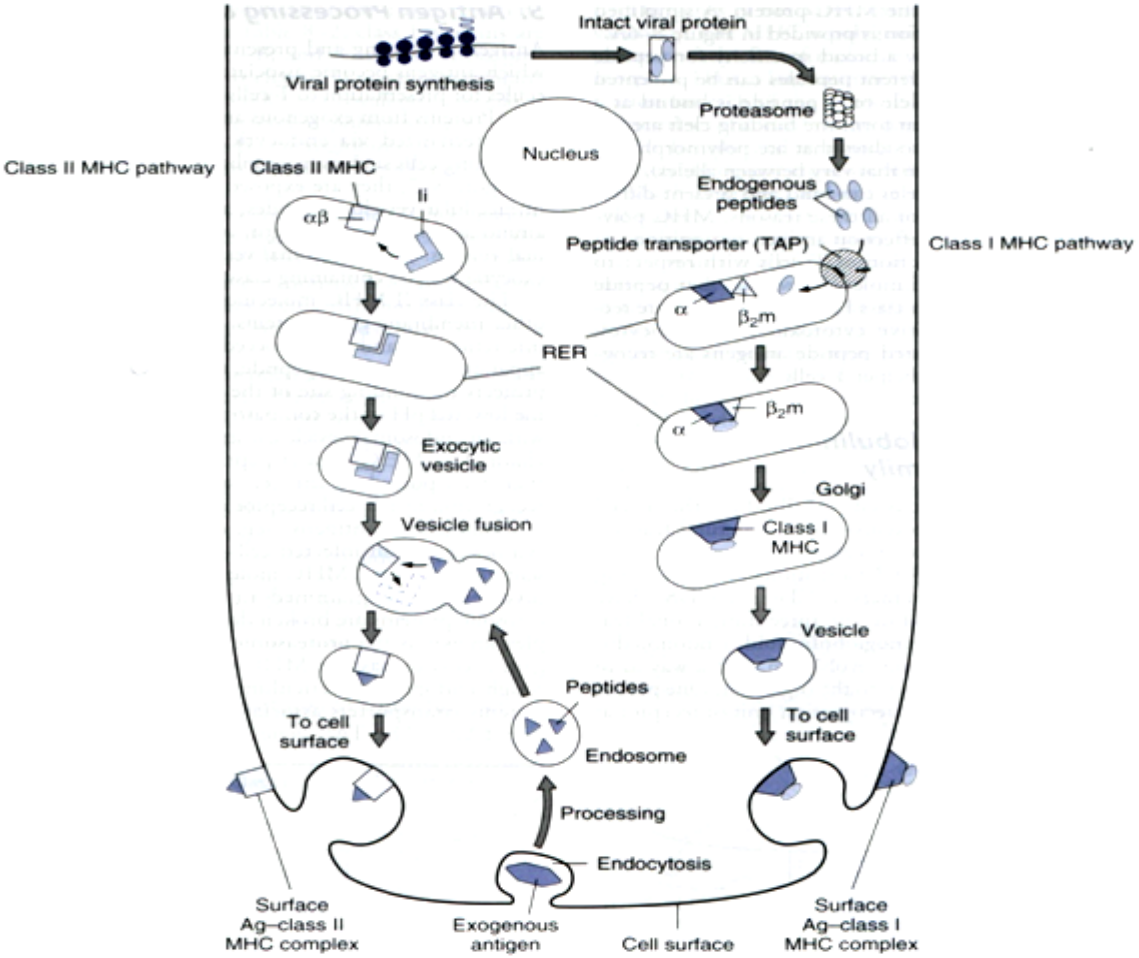


Figure 2.4.2 – Antigen-processing pathways.

ADAPTIVE HUMORAL IMMUNE RESPONCE . IMMUNOGLOBULINS. FLOCULATION AND NEUTRALISATION TESTS.

Theme topicality. The humoral immune response is one of the main reactions for the introduction of the antigens in the human or animal body. Humoral immune response determines the evaluation of the antigens immunogenicity. The result of the humoral immune response is antibodies. One of the basic properties of the antibodies is their specificity. It determines their use for diagnostic purpose for the different antigens determination. Precipitation reaction and its variations are used in medical practice to identify pathogens and toxins (anthrax, meningococcal infection, diphtheria, etc.). The antitoxic serum is used for treatment and prevention of diphtheria, tetanus, botulism, and other diseases.

Primary objective: to be able to use precipitation and agglutination tests, in medical practice and evaluate their results.

QESTIONS FOR DISCUSSION

1. Adaptive humoral immune response: definition, scheme, and phases. Cellular and humoral factors make role in immune response. Antigens are stimulated humoral immune response.
2. Definition, structure, functions, and properties of antibodies. The obtaining and practical use of the antibodies.
3. Immunoglobulins (Ig): types, properties, and functions.
4. Exotoxins, toxoid, antitoxin: definition, properties, flocculation test, and practical use.
5. Neutralisation test: components, purpose of their use, procedure.

PROCEDURE OF PRACTICAL SESSION

Study and record in the protocol demonstration immune serum and antibodies (antitoxic, precipitating)

Antitoxic immune serum and antibodies are used for specific prevention and treatment of several infectious diseases. This will create a passive immunity. Antitoxic serum is prepared by the relevant hyperimmunization of the horses by the toxoid.

Hyperimmunization is made by multiple injections of the toxoid for certain schemes. In the process of immunization, the titre of the serum is detected in the reaction of the flocculation. The quantity of the antitoxic units contained in 1 ml is taken as a titre of the serum. International units (IU) are established for evaluation of the preparations activity in the most antitoxic sera. A unit of product is accepted conditional quantity of the antitoxic serum that neutralizes a certain number of minimum lethal doses of the respective toxin. If the titer of the antibody in horse serum is very high we will make the partial bloodletting and after clotting separate the serum. Then we will filter the obtained antitoxic serum through bacterial filters and expose clean concentration, which is based on removing from the serum ballast proteins (albumin and euglobulins) with the followed concentration of the immunoglobulin. In some cases, fractionation of the sera by ammonium sulfate and processing of the proteolytic enzymes (pepsin) are made.

When using fermentation a titre increases 5–10 times, anaphylactogenic properties of the preparations are reduced. All immune sera before their release are

undergoing the control. Sera should contain a fixed antibody titre, to be sterile and nonpyrogenic. Serum is used for prevention and treatment of the infectious diseases, causative agents of which produce exotoxins (diphtheria, tetanus, gas gangrene, and others). Improvement of the serum preparations completes obtaining an immune gammaglobulins, which is different from purified sera that they have a minimum other proteins. Immune gammaglobulin is prepared of serum of human volunteers that are immunized against various viral or bacterial infections, or with blood serum of the reconvalescents. It is used for treatment and prevention whooping cough, tetanus, influenza, and others. Human immunoglobulin serum is a homogeneous preparation, which rarely causes allergic reactions in humans. Homologous antibodies come faster in the blood and remain longer in it.

Prevention of the complications. When you inject all serum preparations you must study the sensitivity of the patients to the serum that is introduced. The children have serotherapy particularly frequently.

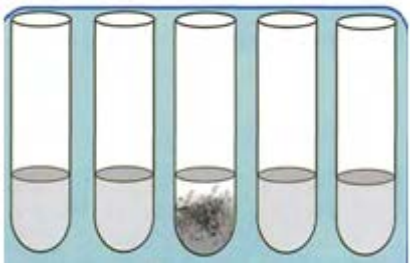
Reintroduction of the serum may lead to increased sensitivity of children organism to this preparation, so skin test is necessary. For this, you must inject intracutaneously 0.1 ml of the serum dilution 1:100. If after 30 minutes blister and hyperaemia have not formed, you can inject serum. Therapeutic dose of serum is introduced fractionally by Bezredko to prevent the development of anaphylactic shock. At first you inject a 1/3 dose, and after 2–3 hours the other quantity of the serum.

Passive immunity is forming after injection of the serum preparations, its duration depends on the speed of the antibodies half-life. This period continuas about 22 days in infants and about 14 days in adults. Homologous immunoglobulin remains in the body a little longer. In this regard, serum preparation is used mainly in serotherapy.

Make the titration of the antitoxic serum by flocculation method and estimate the result.

Scheme of the antitoxic serum titration

Tube number	Component		Result after 20 minutes incubation in the thermostat
	Antitoxic serum	Diphtheria toxin (50 lf in 1 ml)	
1	0.1	2.0	Initial flocculation
2	0.15	2.0	
3	0.2	2.0	
4	0.25	2.0	
5	0.3	2.0	



Flocculation test

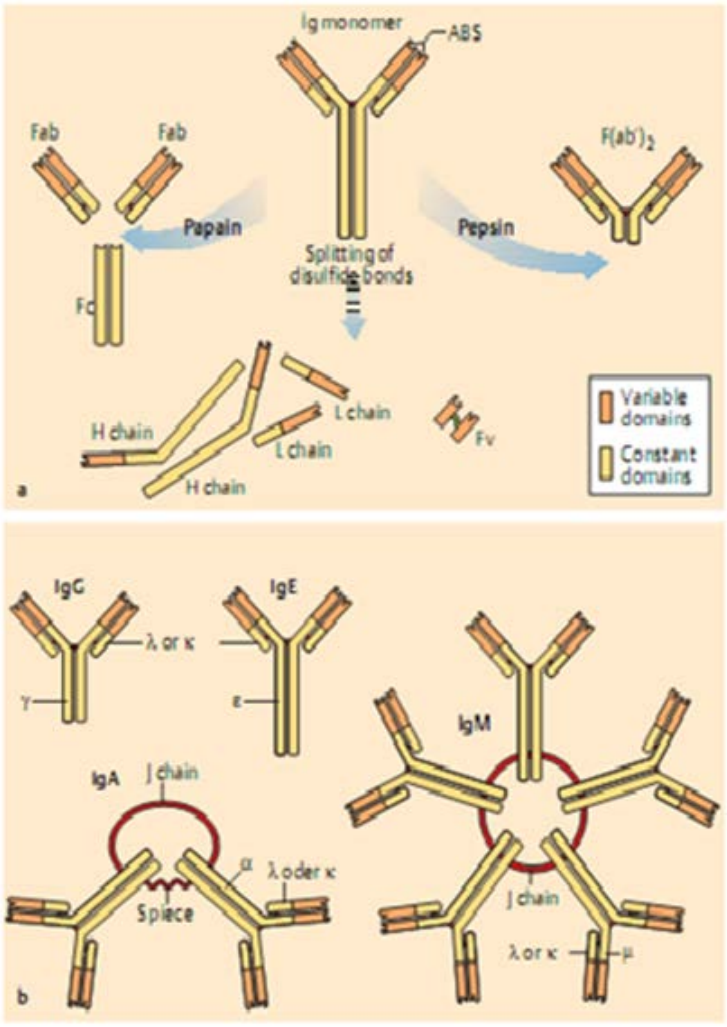
Adaptive humoral immune response. Immunoglobulins (antibodies). Floculation and neutralization tests

Notion	Definition/explanation
Humoral immune response	The reaction arises in the penetration of antigen into the body. It is the chain intercellular and intermolecular interactions terminating the production of the specific antibodies
Antibodies	Specific proteins that are synthesized in the body in response to input (falling) of an antigen
Classes (isotypes) of the antibodies (Ig)	Depending on the structure of Ig (heavy chain types) 5 classes are defined: M, G, A, E, D. Isotype determines the biological properties of antibodies.
The main properties of Ig	1.Heterogenity. 2. Specificity
Antibody specificity	The property to interact only those antigens that stimulate the synthesis of these antibodies
Heterogenity of the antibodies	Large population diversity (10^{10}) binding properties of the antibodies towards antigens
Primary humoral immune responses to antigen	A lag period of approximately 10 days to 2 weeks occurs before a substantial amount of antibody can be detected in the blood following the first (primary) exposure to an antigen. During this delay, the individual could very well experience symptoms of an infection, which could be life-threatening. However, the immune system is actively responding; naive B cells present antigen to T cells, resulting in B-cell activation. The T_H activated B cells multiply, generating a population of cells that recognize the antigen. As some of the activated B cells continue dividing, others differentiate to form plasma cells, which secrete thousands of antibody molecules per second. Each plasma cell generally undergoes apoptosis after several days, but activated B cells

Notion	Definition/explanation
	continue proliferating and differentiating, generating increasing numbers of plasma cells as long as antigen is present. The net result is

	the slow but steady increase in the titre, or concentration, of antibody molecules. Over time, some of the proliferating B cells undergo changes, enhancing the immune response. These include: affinity maturation, class switching, formation of memory cells
The flocculation test. antitoxic serum. Toxoid	Neutralization test as a phenomen on flocculation is a clouding of the tube with a mixture of toxoid (toxin) and antitoxin. It is used in determination of the activity of immune antitoxic serum and a toxoid. Antigen (exotoxins) has a biological (toxic, poisonous) activity, which is neutralized (eliminated, binds) with the help of the antibodies – antitoxin, which contains antitoxic serum. This serologic reaction is

Notion	Definition/explanation
	called neutralization reaction. Antitoxic serum obtained by immunization of horses by a toxoid. It is titrated in vivo (on sensitive to the toxin laboratory animals) or in vitro in reaction flocculation. Activity of the antitoxic sera is measured by international units (IU). 1IU is a dose of the antitoxic serum, which neutralizes fixed amount of the toxin (DLM). Toxoid is obtained by neutralization of the exotoxins by formalin solution (in a mixture of 0.4%) at temperature 40 °C for 4 weeks. Activity of the toxoid is determined in the immune (antigenic) units (AU) in the reaction flocculation. 1 AU is a minimum dose of toxoid, which reacts with 1 IU antitoxic serum and a positive reaction flocculation arises. Antitoxic serum are using for the specific prevention of the infectious diseases, whom causative agents produce exotoxins (diphtheria, tetanus, botulism, gas gangrene), as well as in snakebite. Toxoid is used as vaccines to specific prevention above specified diseases
Neutralization tests	They work because antibodies can neutralize the biological activity of many pathogens and their toxins. For example, combining antibodies against tetanus toxin with a sample of toxin renders the sample harmless to mice because the antibodies have reacted with and neutralized the toxin
Immunoelectrophoresis	It is a combination of two methods – gel electrophoresis and immunodiffusion. This combined method allows the separation molecule by electrophoresis mobility (between anode and cathode) and identification (definition) for their antigenic specificity using homologous immune sera. At first, electrophoresis is making often in hanging gels with a mixture of the antigens. Then, a ditch is cutting the parallel the lines of the antigens movement and the homologous antiserum is brought in it. As a result of a diffusion the antigens and antibodies migrate in the steam of the carrier (gel from the agar) to meet each other, connect in the areas equivalence concentration and form the bow-shaped line of the precipitation. The amount of the line, its position and shape give an idea about the quantitative and qualitative composition of the investigated mixture of the antigens



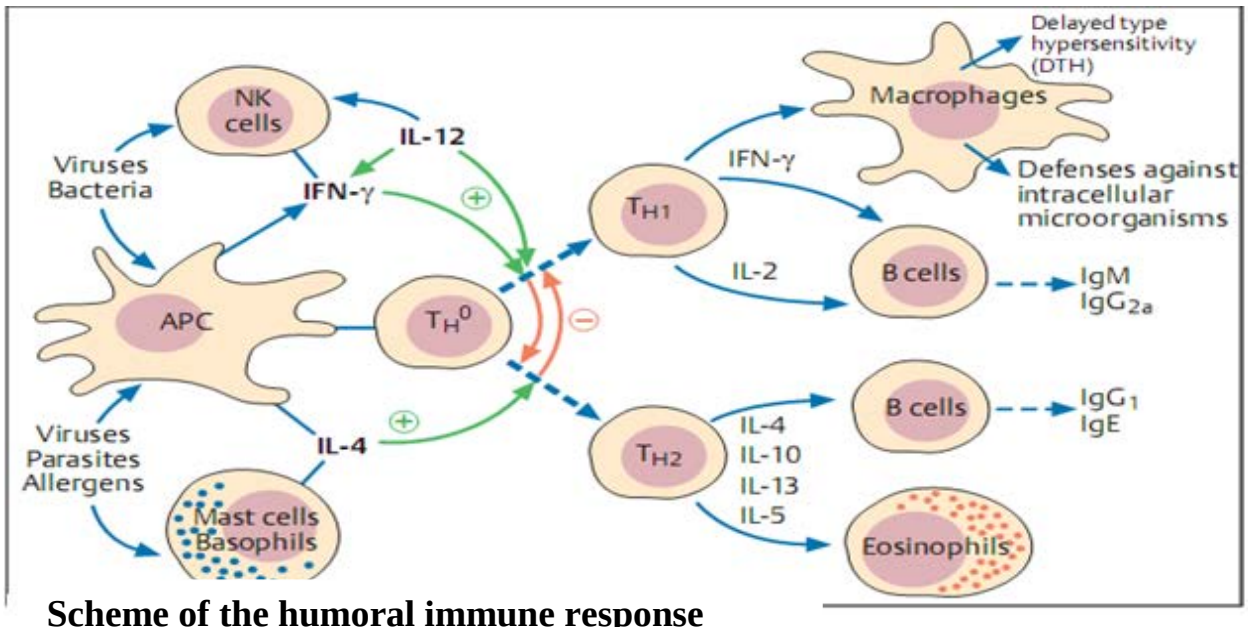
Basic immunoglobulin structures

Immunoglobulin monomers.

The upper half of the figure shows the intact monomer consisting of two L and two H chains. The positions of the disulfide bonds, the variable N-terminal domains, and the antigen-binding site (ABS) are indicated. The lower half of the figure shows the monomers of the individual polypeptide chains as seen following exposure to reducing conditions (which break the disulfide bonds) and denaturing conditions; note that the ABS is lost. Papain digestion produces two monovalent Fab fragments, and one Fc fragment. Following pepsin digestion (right), the Fc portion is fragmented, but the Fab fragments remain held together by disulfide bonds. The F(ab) arm is bivalent (with two identical ABS). Fv fragments comprise a single-chain ABS formed by recombinant technology. These consist of the variable domains of the H and L chains, joined covalently by a synthetic linker peptide.

Classes of immunoglobulins.

IgM, IgD, IgG, IgA, and IgE are differentiated by their respective heavy chains (l, d, c, a, e). IgA (a chain) forms dimers held together by the J (joining) chain; the secretory (S) piece facilitates transport of secretory IgA across epithelial cells, and impairs its enzymatic lysis within secretions. IgM (l chain) forms pentamers with 10 identical ABS; the IgM monomers are held together by J chains. The light chains (k and j) are found in all classes of immunoglobulins.



Scheme of the humoral immune response

SEROLOGICAL TESTS: AGGLUTINATION, PRECIPITATION, COMPLEMENT FIXATION TEST (CFT), TESTS USING LABELED ANTIBODIES AND ANTIGENS (ELISA, IFT, RIA)

Theme topicality. Serological reactions are used in modern medical practice for diagnosis of infectious and other diseases, as well as for assession of treatment and prevention effectiveness.

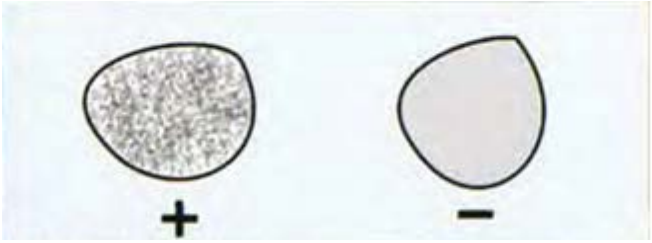
Primary objective: to know the objectives of serological reactions conduction in medical practice and to be able to evaluate their results.

QESTIONS FOR DISCUSSION

1. The main principles and aims of the serological tests in medical practice.
2. Agglutination test and indirect (passive) haemagglutination test (PHAT): definition, mechanism, and practical use.
3. Precipitation reaction: identification, mechanism, types, practical use.
4. Agglutination and precipitation sera: preparation, titration, practical use.
5. Complement fixation test (CFT): the aim of its carrying out, components, mechanisms.
6. Immunofluorescent test (IFT): the variety, aim of its carrying out, components, mechanism.
7. Enzyme linked immunosorbent assay (ELISA): the purpose of its carrying out, components, mechanism.
8. Radioimmunoassay (RIA): the purpose of its carrying out, components, mechanism.

PROCEDURE OF PRACTICAL SESSION

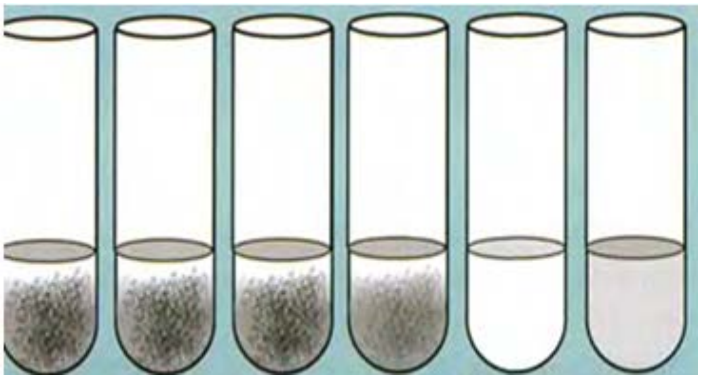
Task 1. Carry out the slide agglutination test. Prepare the smear from agglutinate, stain it with using fischin, examine microscopically.



Put on the slide a drop of the agglutination serum in 1:10 – 1:20 dilution, next to drop a sodium chloride as control. Take a small loop of culture from slant agar and evenly bevelled emulsify it in sodium chloride and serum. A positive agglutination reaction appeared flakes and control drop remains evenly turbid.

test

Carry out the tube agglutination test. Write in the protocol the results and make a conclusion.

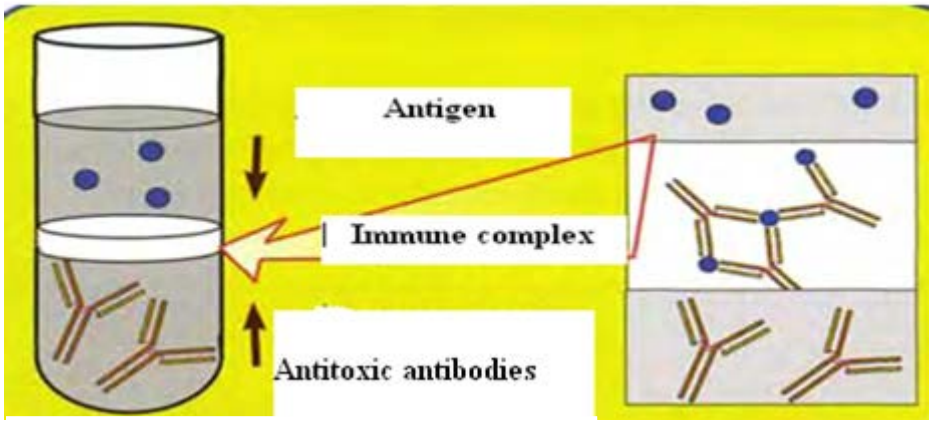


Tube agglutination test

To determine antibodies titre take 7 test tubes. Make the serum dilution from 1:50 to 1:600. In all 7 test tubes, add 2 drops of the brucellosis diagnosticum. Seventh tube is controlled diagnostics (containing saline and diagnostics). Such control is necessary to avoid spontaneous agglutination of culture. Tubes shaken and placed in a thermostat at 37 °C for 2 hours, then leave a day at agglutination test.

Task 3. Carry out the ring precipitation test for detection of the species belonging to the blood spot. Write the reaction in the protocol, estimate the results and make the conclusion.

To determine the species affiliation blood spots carry reaction of ring precipitation. For this put in narrow tube add precipitating serum against human blood protein. Then gently on the wall of the tube, with fine Pasteur pipette, add the extract of the bloodstains. When antibodies bind to soluble antigens that have multiple epitopes, extensive cross-linking of the two types of molecules may occur, forming latticelike insoluble complexes that then precipitate out of solution. This is the basis of precipitation reaction. The reaction was taken into account after incubation in the thermostat tube for 30 minutes at 25 - 26 ° C. When a positive result on the boundary of two liquids formed dense white ring. Spend account the reaction.



Ring precipitate on test

Estimate the result of the complement fixation test (CFT). Write it in the protocol and make a conclusion.

Basic investigation of CFT. Carry out the reaction in 3 tubes. Take the ingredients (antigen, complement, haemolytic serum) and dilute them with physiological solution so that their working dose contained in 0.5 ml. At the same time, prepare 3% sheep erythrocytes suspension. In added series of tubes dilute inactivated (at temperature 56 ºC for 30 minutes) investigated serum. First, mix the investigated serum, antigen, and complement. Keep the mixture at temperature 37 ºC for 40 minutes. After that, add in the tube sensitized haemolytic system (2 ml haemolytic serum + 2 ml of 3% erythrocytes suspensions). Shake again the tubes, put them into the thermostat at 37 ºC. The determination of results of the reaction are made after complete haemolysis in the test tube with the control serum and control antigen.

The result of reaction is determined by the presence or absence of haemolysis in the tube. Reaction is considered to be positive if the haemolysis is fully delayed. In this case the liquid in the tube is colourless, erythrocytes settle to the bottom. If you see full lyses of the erythrocytes and fluid is stained intensively (laky blood), the reaction is negative. The degree of the haemolysis delay is estimated depending on the intensity of liquids staining and size of red sediment on the bottom (++++, + + +, + +, +).

Complement fixation test (CFT) belongs to indirect two-system reactions. Like other serological reactions, it is used for serological diagnosis (determination of specific complement-fixing antibodies in the blood) with a help of the known antigen or determination of the antigens by the known antibodies. As in other serological reactions, in CFT antibody and antigen interact, but complement takes part in it too, that is fixing with antibodies (IgM, IgG), which interact with the antigen. Let us discuss the case when CFT is made for serological diagnosis of infectious diseases. Add known antigen and complement to the patients blood serum. If this serum has specific antibodies to the antigen, the antigen + antibody complex, which binds the complement, will form. But this process is invisible. It was the first system of CFT.

For determination of the complement state (fixed or not fixed) one should add the second system, called haemolytic. Haemolytic system is prepared to consider the reaction and consists of haemolytic serum that contains sheep erythrocytes antibodies (haemolysin) and sheep erythrocytes (SE). Immune reaction of haemolysis in this system occurs in the presence of unfixed component in the first system. Before production reaction, investigated patient's serum is warmed at 56 ºC for 30 minutes to inactivate their own complement, which is located in it. A serum of Guinea pigs is used as a complement to CFT.

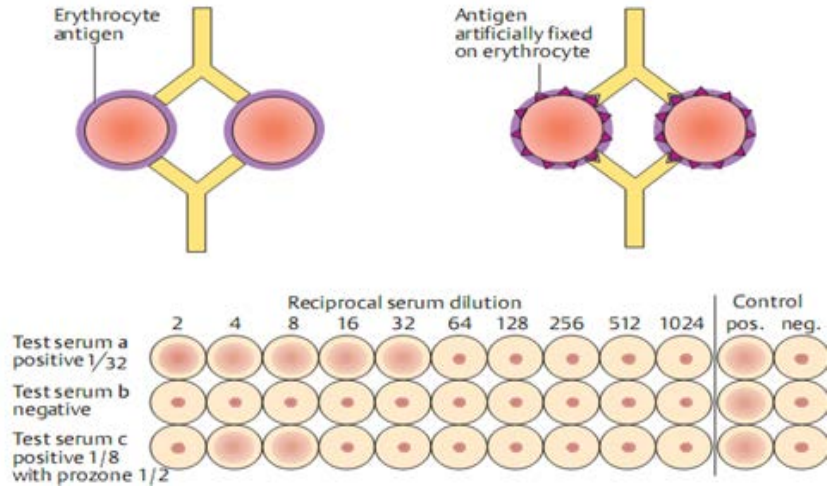
All CFT components, except examined serum, must be preliminary titrated and used in the working dose. In case there are specific antibodies in the sample of serum, antigen + antibody complex is formed. This complex fixes complement through Fc-fragment of the antibodies, but it is not visually noticeable. We add the haemolytic system to the mixture and if complement is fixed in the first system, there is no haemolysis in the second system and reaction is positive – sheep erythrocytes precipitate.

In case there are no antibodies in the studied serum, complement remains unfixed in the first system. When we add second (haemolytic) system we see haemolysis of erythrocytes, which indicates that the CFT is negative. CFT is used for diagnosis of syphilis, tuberculosis, brucellosis, candidiasis, influenza, and other diseases.

Study and write in the protocol the demonstration with the conclusions.

Gel precipitation test

This technique facilitates assignment of antigens (violet) to a certain test antibody (yellow), or vice versa. To determine the toxigenicity of the diphtheria culture the strip filter paper soaked serum containing 1 ml of the 500 IU are placed on the dish with agar and then make the inoculation the diphtheria culture as a plague at 1 cm from the edge of the strip. If culture produces exotoxins, it diffuse in nutrient environment. Where they meet lines of precipitation (known as precipitin bands) develop, indicating immune complex formation. Three independent precipitin bands form, indicating that the antibodies differentiate three different epitopes on three different antigens.



Passive (indirect) hemagglutination test (PHAT)

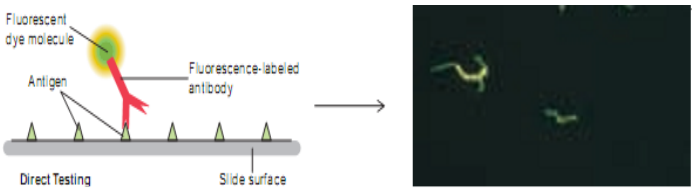
Haemagglutination test is based on the principle that erythrocytes cross-linked by antibodies settle to the bottom of the microtitre plate wells inmate-like aggregates, whereas nonagglutinated erythrocytes collect at the lowest point of the wells to form a single “button” in the middle. The test sera are first pipetted into the wells at the indicated dilutions, then the erythrocyte suspension is added. Nonspecific agglutination is prevented by addition of an irrelevant protein. The test can be carried out using erythrocyte antigens (above left). Alternatively, other antigens can be fixed to the erythrocyte surface and the agglutination monitored (above right). The so-called “prozone” phenomenon results from nonspecific blocking mechanisms present in sera which has not been sufficiently diluted.

Agglutination serum is obtained by immunization of laboratory animals by cultures of the bacteria. In the rabbit ear vein boundary injected 0.5 ml suspension of killed microbe density of 1 billion microbial bodies in 1 ml. Injections are often at intervals of 5-6 days. The dose is increased each time. On the 10th day after the last injection of the same veins, blood is taken. Serum will taking after clotting. It contains antibodies against the bacteria, which conducted immunization. The titre of agglutination serum is the largest delution causing a phenomenon of

specific flocculation. Agglutination typical mono-receptors serum is obtained by immunization of animals relevant antigens. They are used for serological identification of bacteria (salmonella, shigella, etc.).

Direct immunofluorescence can be used for in-vivo detection of antibodies, complement, viruses, fungi, bacteria, or other immune factors present within patient cells and tissues. For this purpose tissue sections, or cell preparations, are treated with specific antibodies (anti-sera) which have been labeled with a fluorochrome. Antigen-antibody reactions can thus be detected using a fluorescence microscope. The fluorochrome absorbs light of a certain wavelength (e.g., UV light), and emits the light energy in the form of light at a different (visible) wavelength. The fluorochrome fluorescein isothiocyanate (FITC), which absorbs UV light and emits it as green light, is used most frequently (caution: bleaches out quickly).

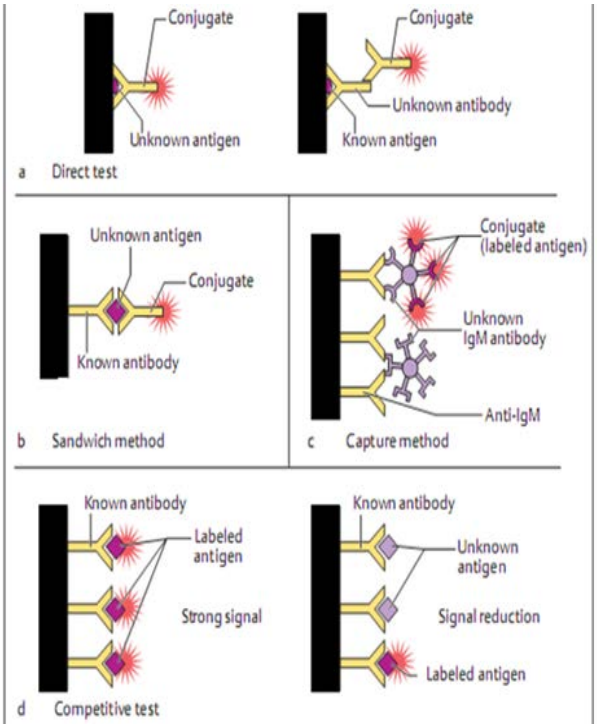
Indirect immunofluorescence and enzyme histology. In this technique the specific or "first" antibody can be unlabeled. The antigen antibody complexes are then detected using a labeled or "second" antibody, directed against the first antibody. Indirect immunofluorescence can be used for the qualitative and quantitative analysis of antibodies directed against particular microbial antigens, or self-tissue antigens, within a patients serum. In the quantitative test, the antigen is fixed in a well or to a tissue section on a slide. The patient sample is repeatedly diluted by a factor of two and added to the antigen or section then rendered visible with a labeled antiantibody.



Direct immunofluorescence test

Radioimmunological and enzyme linked immunosorbent assay.

For solid phase tests both the antigen and antibody are bound to a solid phase (e.g., plastic surface). Various methods are then used to detect any interaction between the antigen and antibody. In the direct test (a) an immobilized, unknown, antigen can be detected using a fluorescent-labeled antibody. If the immobilized antigen is known, this test method can also be used to detect an antibody bound to the antigen. In the sandwich method (b) a known antibody is immobilized. Detection of antibody-antigen binding is then performed using a second, labeled antibody which interacts with the antigen at a different site. The capture method (c) can be used to detect any antigen, for instance, IgM antibodies. First, anti-IgM antibodies are immobilized, then serum containing IgM is added to them. The bound IgM can then bind a foreign antigen (e.g., virus). The detection procedure next makes use of either the labeled foreign antigen or a specific, additionally labeled, antibody which binds to the bound antigen but not to the plastic bound antibody.



Basic solid phase test types

In the competition or competitive inhibition test (d) antibodies are immobilized, and labeled antigens are then bound to them. An unlabeled (unknown) antigen is added, which competes with the labeled antigen. The level of interaction between the antibody and the unknown antigen is then determined by measuring attenuation of the signal.

SEROLOGICAL TESTS: AGGLUTINATION, PRECIPITATION, COMPLEMENT FIXATION TEST (CFT), TESTS USING LABELED ANTIBODIES AND ANTIGENS (ELISA, IFT, RIA)

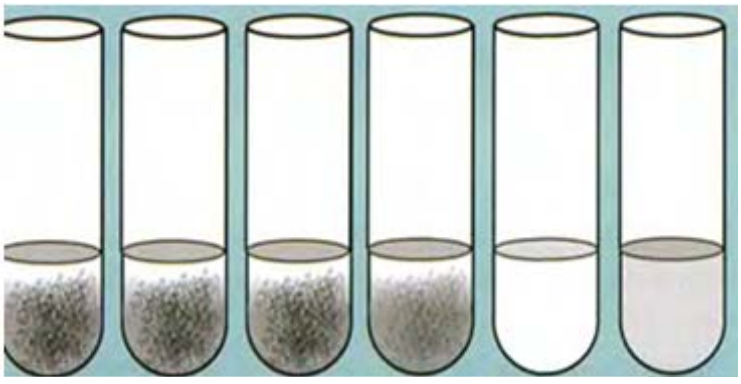
Theme topicality. Serological reactions are used in modern medical practice for diagnosis of infectious and other diseases, as well as for assession of treatment and prevention effectiveness.

Primary objective: to know the objectives of serological reactions conduction in medical practice and to be able to evaluate their results.

QESTIONS FOR DISCUSSION

9. The main principles and aims of the serological tests in medical practice.
10. Agglutination test and indirect (passive) haemagglutination test (PHAT): definition, mechanism, and practical use.
11. Precipitation reaction: identification, mechanism, types, practical use.
12. Agglutination and precipitation sera: preparation, titration, practical use.
13. Complement fixation test (CFT): the aim of its carrying out, components, mechanisms.
14. Immunofluorescent test (IFT): the variety, aim of its carrying out, components, mechanism.
15. Enzyme linked immunosorbent assay (ELISA): the purpose of its carrying out, components, mechanism.
16. Radioimmunoassay (RIA): the purpose of its carrying out, components, mechanism.

Carry out the tube agglutination test. Write in the protocol the results and make a conclusion.



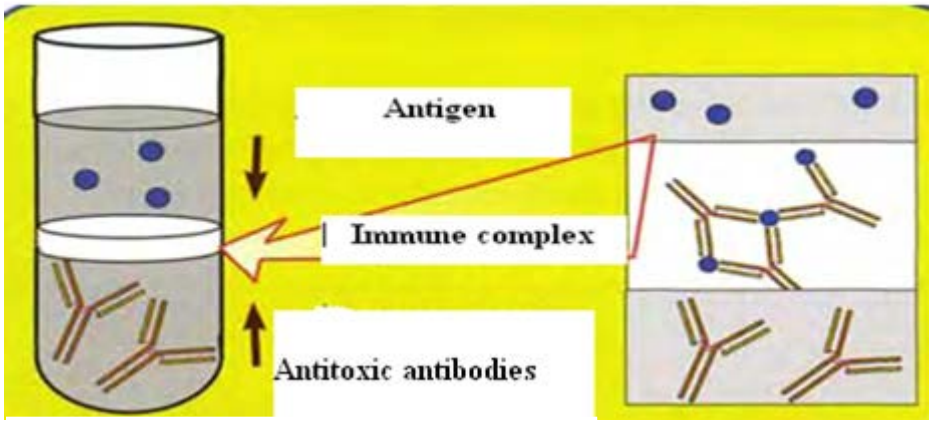
Tube agglutination test

To determine antibodies titre take 7 test tubes. Make the serum dilution from 1:50 to 1:600. In all 7 test tubes, add 2 drops of the brucellosis diagnosticum. Seventh tube is controlled diagnostics (containing saline and diagnostics). Such control is necessary to avoid spontaneous agglutination of culture.

Tubes shaken and placed in a thermostat at 37 °C for 2 hours, then leave a day at room temperature, then making accounting results agglutination test.

Carry out the ring precipitation test for detection of the species belonging to the blood spot. Write the reaction in the protocol, estimate the results and make the conclusion.

To determine the species affiliation blood spots carry reaction of ring precipitation. For this put in narrow tube add precipitating serum against human blood protein. Then gently on the wall of the tube, with fine Pasteur pipette, add the extract of the bloodstains. When antibodies bind to soluble antigens that have multiple epitopes, extensive cross-linking of the two types of molecules may occur, forming latticelike insoluble complexes that then precipitate out of solution. This is the basis of precipitation reaction. The reaction was taken into account after incubation in the thermostat tube for 30 minutes at 25 - 26 ° C. When a positive result on the boundary of two liquids formed dense white ring. Spend account the reaction.



Ring precipitation test

Estimate the result of the complement fixation test (CFT). Write it in the protocol and make a conclusion.

Basic investigation of CFT. Carry out the reaction in 3 tubes. Take the ingredients (antigen, complement, haemolytic serum) and dilute them with physiological solution so that their working dose contained in 0.5 ml. At the same time, prepare 3% sheep erythrocytes suspension. In added series of tubes dilute inactivated (at temperature 56 ºC for 30 minutes) investigated serum. First, mix the investigated serum, antigen, and complement. Keep the mixture at temperature 37 ºC for 40 minutes. After that, add in the tube sensitized haemolytic system (2 ml haemolytic serum + 2 ml of 3% erythrocytes suspensions). Shake again the tubes, put them into the thermostat at 37 ºC. The determination of results of the reaction are made after complete haemolysis in the test tube with the control serum and control antigen.

The result of reaction is determined by the presence or absence of haemolysis in the tube. Reaction is considered to be positive if the haemolysis is fully delayed. In this case the liquid in the tube is colourless, erythrocytes settle to the bottom. If you see full lyses of the erythrocytes and fluid is stained intensively (laky blood), the reaction is negative. The degree of the haemolysis delay is estimated depending on the intensity of liquids staining and size of red sediment on the bottom (++++, + + +, + +, +).

Complement fixation test (CFT) belongs to indirect two-system reactions. Like other serological reactions, it is used for serological diagnosis (determination of specific complement-fixing antibodies in the blood) with a help of the known antigen or determination of the antigens by the known antibodies. As in other serological reactions, in CFT antibody and antigen interact, but complement takes part in it too, that is fixing with antibodies (IgM, IgG), which interact with

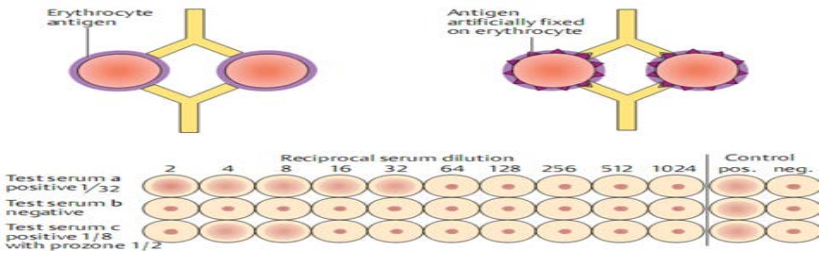
the antigen. Let us discuss the case when CFT is made for serological diagnosis of infectious diseases. Add known antigen and complement to the patients blood serum. If this serum has specific antibodies to the antigen, the antigen + antibody complex, which binds the complement, will form. But this process is invisible. It was the first system of CFT. For determination of the complement state (fixed or not fixed) one should add the second system, called haemolytic. Haemolytic system is prepared to consider the reaction and consists of haemolytic serum that contains sheep erythrocytes antibodies (haemolysin) and sheep erythrocytes (SE). Immune reaction of haemolysis in this system occurs in the presence of unfixed component in the first system. Before production reaction, investigated patient's serum is warmed at 56 ºC for 30 minutes to inactivate their own complement, which is located in it. A serum of Guinea pigs is used as a complement to CFT. All CFT components, except examined serum, must be preliminary titrated and used in the working dose. In case there are specific antibodies in the sample of serum, antigen + antibody complex is formed. This complex fixes complement through Fc-fragment of the antibodies, but it is not visually noticeable. We add the haemolytic system to the mixture and if complement is fixed in the first system, there is no haemolysis in the second system and reaction is positive – sheep erythrocytes precipitate. In case there are no antibodies in the studied serum, complement remains unfixed in the first system. When we add second (haemolytic) system we see haemolysis of erythrocytes, which indicates that the CFT is negative. CFT is used for diagnosis of syphilis, tuberculosis, brucellosis, candidiasis, influenza, and other diseases.

Study and write in the protocol the demonstration with the conclusions.

Gel precipitation test

This technique facilitates assignment of antigens (violet) to a certain test antibody (yellow), or vice versa. To determine the toxigenicity of the diphtheria culture the strip filter paper soaked serum containing 1 ml of the 500 IU are placed on the dish with agar and then make the inoculation the diphtheria culture as a plague at 1 cm from the edge of the strip. If culture produces exotoxins, it diffuse in nutrient environment. Where they meet lines of precipitation (known as precipitin bands) develop, indicating immune complex formation. Three independent precipitin bands form, indicating that the antibodies differentiate three different epitopes on three different antigens.

Haemagglutination test is based on the principle that erythrocytes cross-linked by antibodies settle to the bottom of the microtitre plate wells inmate-like aggregates, whereas nonagglutinated erythrocytes collect at the lowest point of the wells to form a single "button" in the middle.



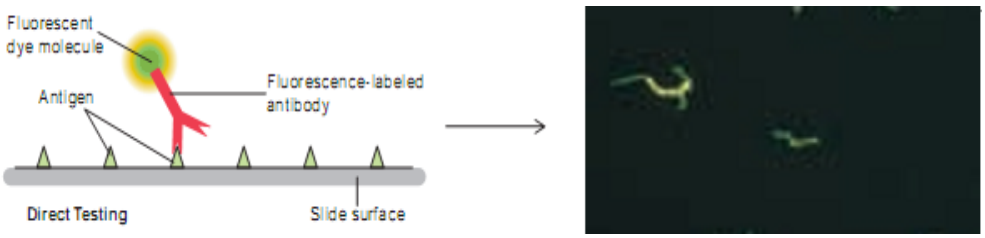
Passive (indirect) hemagglutination test (PHAT)

The test sera are first pipetted into the wells at the indicated dilutions, then the erythrocyte suspension is added. Nonspecific agglutination is prevented by addition of an irrelevant protein. The test can be carried out using erythrocyte antigens (above left). Alternatively, other antigens can be fixed to the erythrocyte surface and the agglutination monitored (above right). The so-called “prozone” phenomenon results from nonspecific blocking mechanisms present in sera which has not been sufficiently diluted.

Agglutination serum is obtained by immunization of laboratory animals by cultures of the bacteria. In the rabbit ear vein boundary injected 0.5 ml suspension of killed microbe density of 1 billion microbial bodies in 1 ml. Injections are often at intervals of 5-6 days. The dose is increased each time. On the 10th day after the last injection of the same veins, blood is taken. Serum will taking after clotting. It contains antibodies against the bacteria, which conducted immunization. The titre of agglutination serum is the largest delution causing a phenomenon of specific flocculation. Agglutination typical mono-receptors serum is obtained by immunization of animals relevant antigens. They are used for serological identification of bacteria (salmonella, shigella, etc.).

Direct immunofluorescence can be used for in-vivo detection of antibodies, complement, viruses, fungi, bacteria, or other immune factors present within patient cells and tissues. For this purpose tissue sections, or cell preparations, are treated with specific antibodies (anti-sera) which have been labeled with a fluorochrome. Antigen-antibody reactions can thus be detected using a fluorescence microscope. The fluorochrome absorbs light of a certain wavelength (e.g., UV light), and emits the light energy in the form of light at a different (visible) wavelength. The fluorochrome fluorescein isothiocyanate (FITC), which absorbs UV light and emits it as green light, is used most frequently (caution: bleaches out quickly).

Indirect immunofluorescence and enzyme histology. In this technique the specific or “first” antibody can be unlabeled. The antigen antibody complexes are then detected using a labeled or “second” antibody, directed against the first antibody. Indirect immunofluorescence can be used for the qualitative and quantitative analysis of antibodies directed against particular microbial antigens, or self-tissue antigens, within a patients serum. In the quantitative test, the antigen is fixed in a well or to a tissue section on a slide. The patient sample is repeatedly diluted by a factor of two and added to the antigen or section then rendered visible with a labeled antiantibody.

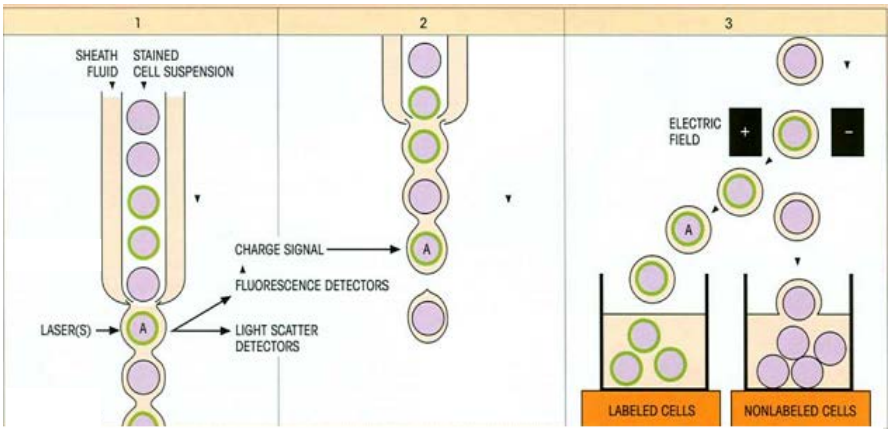


Direct immunofluorescence test

Radioimmunological and enzyme linked immunosorbent assay.

For solid phase tests both the antigen and antibody are bound to a solid phase (e.g., plastic surface). Various methods are then used to detect any interaction between the antigen and antibody. In the direct test (a) an immobilized, unknown, antigen can be detected using a fluorescent-labeled antibody. If the immobilized antigen is known, this test method can also be used to detect an antibody bound to the antigen. In the sandwich method (b) a known antibody is immobilized. Detection of antibody-antigen binding is then performed using a second, labeled antibody which interacts with the antigen at a different site. The capture method (c) can be used to detect any antigen, for instance, IgM antibodies. First, anti-IgM antibodies are immobilized, then serum containing IgM is added to them. The bound IgM can then bind a foreign antigen (e.g., virus). The detection procedure next makes use of either the labeled foreign antigen or a specific, additionally labeled, antibody which binds to the bound antigen but not to the plastic bound antibody. In the competition or competitive inhibition test (d) antibodies are immobilized, and labeled antigens are then bound to them. An unlabeled (unknown) antigen is added, which competes with the labeled antigen. The level of interaction between the antibody and the unknown antigen is then determined by measuring attenuation of the signal.

The fluorescence-activated cell sorter (FACS) was developed by the Hembergs and their colleagues to quantify the surface molecules on individual white cells by their reaction with fluorochrome labeled monoclonal antibodies and tow the signals sogenerated to separate cells of defined phenotype from a heterogeneous mixture.



The fluorescence-activated cell sorter principle

In this elegant but complex machine, the fluorescent cells are made to flow obediently in a single stream past a laser beam. Quantitative measurement of the fluorescent signal in a suitably placed photomultiplier tube relays a signal to the cell as it emerges in a single droplet; the cell becomes charged and can be separated in an electric fie1d.

Antigens used in CFT chemically may be protein or polysaccharide microorganisms antigens, bacterial cells, viruses, extracts of organs and tissues.

Complement is fresh or lyophilized serum of Guinea pigs that is diluted in 1:10. Complement is titrated on the day of CFT carry out.

Haemolytic serum is prepared by erythrocytes immunization of the rabbits and sheep. It is titrated and used on the correct titre with sheep erythrocytes. 3% suspension is prepared of the sheep erythrocytes in the isotonic sodium chloride solution.

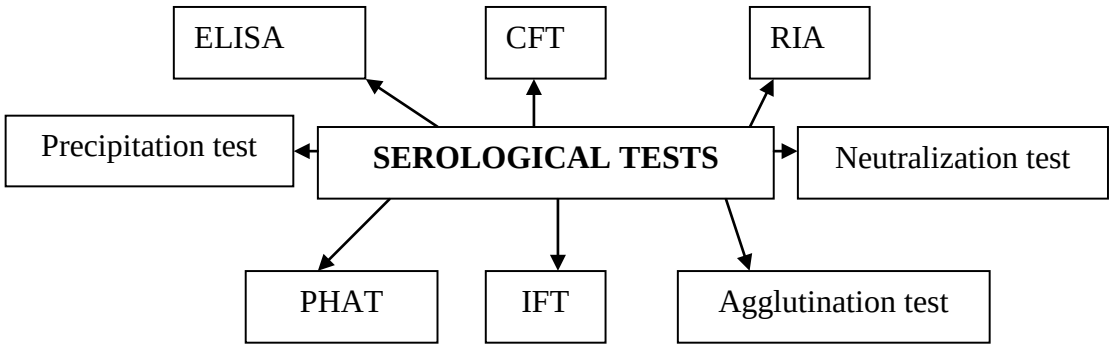
Serological tests: agglutination, precipitation, complement fixation test (CFT), tests using labeled antibodies and antigens – ELISA, IFT, RIA

Notion	Definition/explanation
Practical use of the agglutination test	1. In serological test for detection of the specific antibodies formed in the serum or other body fluids by the infectious diseases and other pathological conditions. 2. For identification of the bacterial antigens in the bacteriological method
Types of the agglutination test	1. Slide agglutination test is made on the glass or porcelain plates with a smooth surface. 2. Tube agglutination test is made in the test tube or in the polisterol hole in plates with consecutive dilution of the serum. In every tube (hole) the same amount of antigen is contributed. The titre of the antibody is detected
Precipitation test mechanism	Interaction of a soluble antigen (precipitinogen) and antibodies (precipitin) in the presence of electrolyte (0.85% solution of NaCl). An aggregation of the antigen makes opacity of transparent liquid (precipitate). Deposition from solution complexes of the antigen-antibody become in equivalent ratio in the range of the interacting molecules concentrations
Varieties of precipitation test (PT)	PT happens when mixing solutions of antigen and antibody or layers of one component to another. In the last variants precipitate take a ring form on the border of the two reagents. Therefore, this reaction was named ring-precipitation tets. Reaction is making in a narrow test tube with 0.1–0.5 ml reagent. For determine the antigens is used methods of precipitation, or diffusion in agar or agarose gels. Scope of the reaction is that the antigen

Notion	Definition/explanation
	and antibody diffuse in a gel pores to meet each other, interact with each other and form a complex that is deposited in a line of precipitation. PT is more sensitive than the agglutination test and can detect a specific antigen in very low concentrations. Therefore, precipitation test is mainly used to determine the antigen. Diagnostic precipitation serum is prepared by immunization of the rabbits by a solution antigen on a special scheme. The titre is the biggest dilution of the antigen, which is formed precipitate
Practical use of the PT	PT is used for the diagnosis of a number of infectious diseases - anthrax, plague, tularemia, epidemic cerebrospinal meningitis and others. Extracts from affected organs, and other liquor are used as the antigens, and diagnostic precipitating serum is used as antibodies (precipitins). PT is also used to study the antigenic structure of the bacteria. In addition, it is used to determine the species proteins, including proteins of blood
Immune-enzyme analysis (ELISA)	Immunoassay analysis (ELISA) is based on the detection of the antigens with the appropriate (specific) antibodies that are binding with a dot-enzyme (horseradish peroxidase or alkaline phosphatase). After connecting with the labeled antigen, substrate (chromogen), such as H_2O_2, is added in the mixture of the serum. Substrate enzyme splits

	and colour of the reacting mixture changes. The intensity of reaction staining is directly proportional to the number of antigen and antibodies molecules, which took part in the reaction. Solid phase of ELISA is the most common method, when one of the components of the immune response (antigen or antibody) is retained on the solid medium, such as holes in the board of the plastic surface. Add the antibodies of the patient's serum, antiglobulin serum labeled by enzyme, substrate (chromogen) for enzymes in the holes of the plate with retained antigen. Each time after addition of the following component, remove the unfixing reagents from the holes by means of washing. If the result is positive, the colour will change. Solid phase vehicle can sensitize an antigen and an antibody. In this case, search antigen is added in the holes with the retained antibody, than enzyme labeled antiserum, and chromogen for the enzyme are added

SEROLOGICAL TESTS



CELLULAR IMMUNE RESPONSE. IMMUNOTOLERANCE. REGULATION OF THE IMMUNE RESPONSE.

Theme topicality. The adaptive immune reactions are led by the infectious diseases that are caused by intracellular parasites, cancer, autoimmune diseases, delayed hypersensitivity, and in transplantation immunity. Knowledge of the mechanisms and assessment of the cell type immune response are necessary for doctor to understand the pathogenesis, diagnosis, and treatment of these states. Artificial immunological tolerance is used for organ transplantation. There are many situations in medical practice when a doctor has to intervene in the regulation of patients' immune responses (stimulation or depression and knowledge of the mechanisms of these processes).

Primary objective: to be able to estimate the state of cellular immunity for the detection of immunodeficiency.

QESTIONS FOR DISCUSSION

1. Adaptive cellular immune response: definition and varieties of the adaptive cellular immune response. Antigens are caused the cellular immune response.
2. Cytotoxic type of the cellular immune response: skim, cytokines, effector cells, mechanisms of action, apoptosis.
3. Diseases in which the leading role belongs to the cytotoxic type of the immune response.
4. Delayed type of the cellular immune response: skim, cytokines, effector cells, mechanism of action.
5. Diseases in which the leading role belongs to the delayed type of the immune response.
6. Primary and secondary immune response. Memory cells, practical value.
7. Immunotolerance: definition, types, mechanisms of action, practical use.
8. Regulation of the immune responses in the body: exhaustive factors and mechanisms.

PROCEDURE OF PRACTICAL SESSION

Examine and record in the protocol antigenic preparations indicating the composition and purpose of use:
1) vaccine BCG, vaccine brucelosis, anthracis, live plague vaccine;
2) tuberculin, antraxin, lepromin, brucellin.

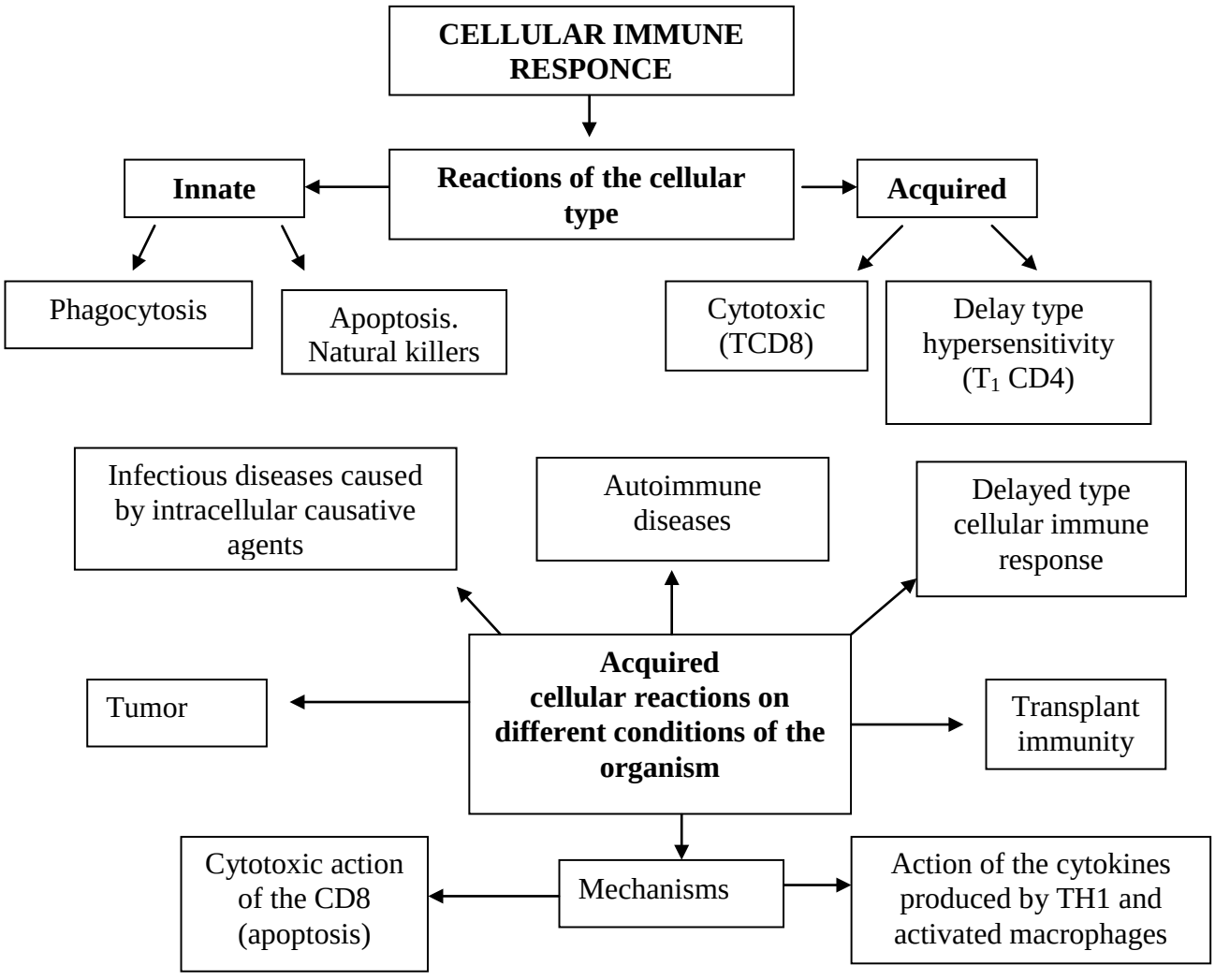
Cellular immune response. Immunotolerance. Regulation of the immune response

Notion	Definition/explanation
Cellular immune response	Acquired response. The effectors of this immune response are
	T cytotoxic lymphocytes (CD8 killers), T-lymphocyte of the delayed type of the hypersensitivity (DTH) and activated macrophages
Types of the cellular immune response	Cytotoxic (TCD8) and delay type hypersensitivity (DTH) (T_1 CD4)
The major cytokines of the cellular immune response	INF-γ, IL-2, TNF-α, IL-12
Migration inhibitory factor (MIF)	Migration inhibitory factor of the macrophages was the first cytokine the activity of which was identified. MIF is produced by the T-lymphocytes under the influence of endotoxin and some
	cytokines. MIF stimulates the production of the hydrogen peroxide, nitrogen, IL-1a, IL-6 and INF-ά by macrophages. MIF increases the expression of molecules HLA II class on the macrophages and stimulates killing of the microorganisms and tumor cells. Besides the MIF synergistic with IL-2 stimulates the proliferation of the T-lymphocytes, blocks the antiinflammation action of glucocorticoids
Delayed dermal hypersensitivity reaction	The classic example of a delayed type hypersensitivity (DTH) reaction is the tuberculin reaction (Mantoux test in humans). It was one of the first specific cell-mediated immune responses to be identified as early as the 1940s in guinea pigs. The response is specific for MHC class II antigens and is CD4 T cell-dependent. In some cases, especially during active viral infections, a DTH reaction

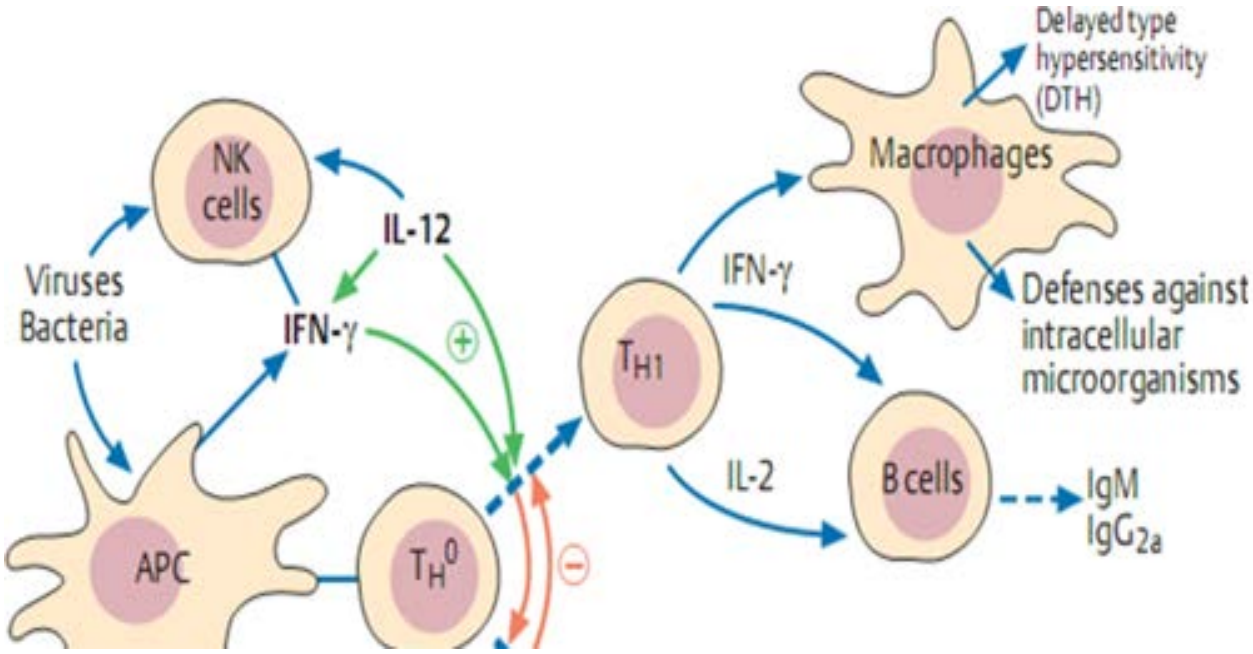
	is transiently observed and is mediated by CD8 T cells. The simplest way to elicit a DTH reaction is to introduce a diagnostic protein, obtained from the pathogen, into the skin. The test reaction will only develop should continuously activated T cells be present within the host, since only these cells are capable of migrating to dermal locations with in 24–48 hours. If no activated T cells are present, reactivation within the local lymph nodes must first take place, and hence migration into the dermis will require more time. By this time the small amount of introduced diagnostic peptide, or protein, will have been digested or will have decayed and thus will no longer be present at the injection site in the quantity required for induction of a local reaction. A positive delayed hypersensitivity reaction is, therefore, an indicator of the presence of activated T cells. The absence of a reaction indicates either that the host had never been in contact with the antigen, or that the host no longer possesses activated T cells. In the case of tuberculosis, a negative skin test can indicate that no more antigen or granuloma tissue is present, or that the systemic immune response is massive and the pathogen is spread throughout the body. In the latter case, the amount of diagnostic protein used is normally insufficient for the attraction of responsive T cells to the site of injection, and as a consequence no measurable reaction becomes evident (so that the Mantoux test may be negative in Landouzy sepsis or miliary tuberculosis). DTH reactions provide a diagnostic test for tuberculosis (Mantoux test), leprosy (lepromin test), and Boeck's sarcoid (Kveim test). However, these dermal reactions may disappear in those patients that are immunosuppressed or infected with measles or AIDS
Immunological tolerance	Immunological tolerance describes the concept that the immune system does not normally react to autologous structures, but maintains the ability to react to foreign antigens. Tolerance is acquired, and can be measured as the selective absence of immunological reactivity against specified antigens. T-cell tolerance, as defined by a lack of immune reactivity can be due to a number of processes: 1. Negative selection in the thymus (referred to as deletion). 2. A simple lack of reactivity to antigen (self or non-self) as a result of the antigen having not been present in the secondary

Notion	Definition/explanation
	1. lymphoid organs in a sufficient quantity or for a sufficient amount of time. 2. An excessive stimulation of T-cells resulting from the ubiquitous presence of sufficient antigen resulting in T cell exhaustion. 3. It may also be possible that T cells can become temporarily "anergized" by partial or incomplete antigen stimulation. As a general rule, self-reactive (autoimmune) B cells are not generally deleted by negative selection and can therefore be present in the periphery. Exceptions to this rule include B cells specific for membrane-bound self-determinants, some of which are deleted or anergized. B cells react promptly to antigens, even self-antigens, which are arranged repetitively. However, they only react to soluble monomeric antigens if they additionally receive T cell help. Thus, B-

	cell nonreactivity largely results from a lack of patterned antigen presentation structures or as a result of T-cell tolerance
Immunological memory	Immunological memory is usually defined by an earlier and better immune response, mediated by increased frequencies of specific B or T cells as determined by in vitro or adoptive transfer experiments. B-cell immunological memory is more completely described as the ability to mediate protective immunity by means of increased antibody concentrations. Higher frequencies of specific B and T lymphocytes alone, appears to only provide limited or no protection. Instead, immunological protection requires antigen-dependent activation of B and T cells, which then produce antibodies continuously or can rapidly mediate effector T functions and can rapidly migrate into peripheral tissues to control virus infections. Usually the second time a host encounters the same antigen its immune response is both accelerated and augmented. This secondary immune response is certainly different from the primary response, however, it is still a matter of debate as to whether these parameters alone correlate with immune protection. It is not yet clear whether the difference between a primary and secondary immune response results solely from the increased numbers of antigen-specific B and T cells and their acquisition of "memory qualities", or whether immune protection is simply due to continuous antigen-induced activation

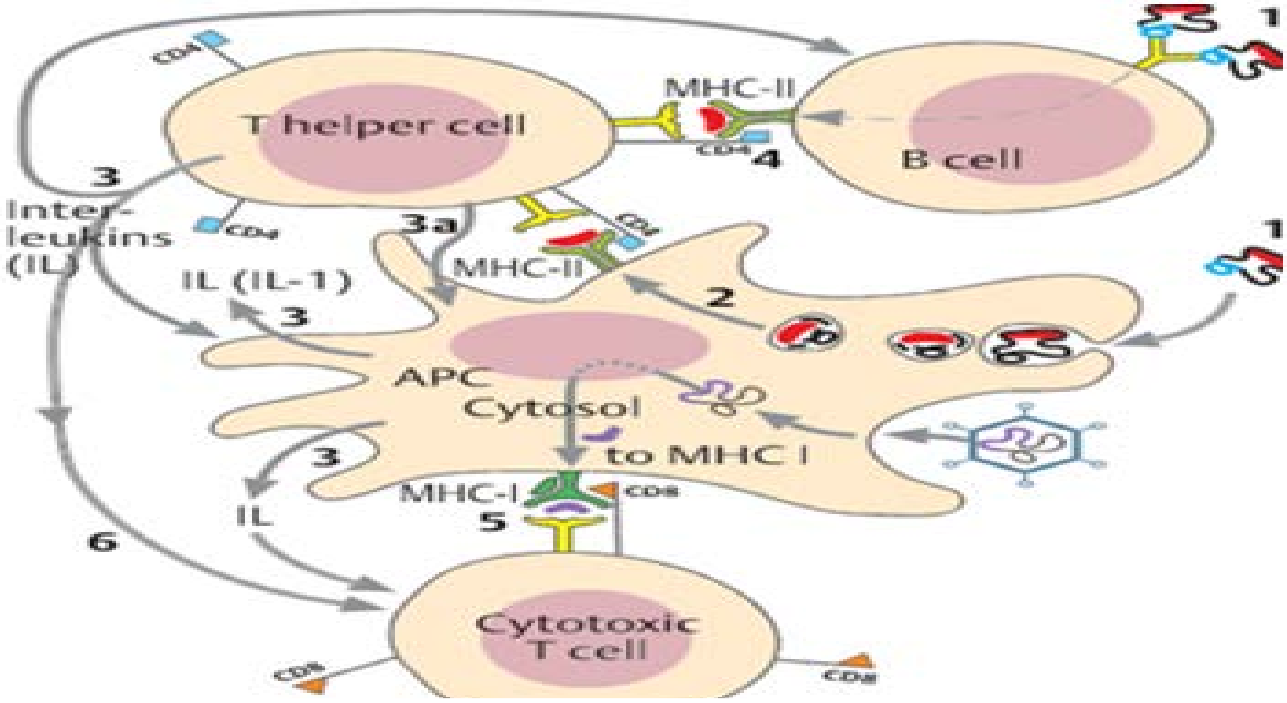


Cellular immune response



Delayed type cellular immune response

TH1 and TH2 cells are derived from a TH0 cell, and undergo differentiation in the presence of help derived from cytokines, DC, macrophages, and other cell types. TH1 cells are activated by IL-12 and IFNγ and are inhibited by IL-4; whilst for TH2 cells the reverse is true. Viruses and bacteria (particularly intracellular bacteria) can induce a TH1 response by activating natural killer cells. In contrast, allergens and parasites induce a TH2 response via the release of IL-4. However, the strong in vitro differentiation of CD4+ T cells intoTH1–TH2 subsets is likely to be less sharply defined in vivo.



Cytotoxic type of the cellular immune response

Mature CD8 T cells perform the biologically important function of lysing target cells. Target cell recognition involves the association of MHC class I structures with peptides normally derived from
endogenous sources, i.e., originating in the cells themselves or synthesized within them by intracellular parasites. Induction of cytotoxic CD8 T cell response often does not require cells or only requires these cells indirectly. However, should the antigen stimulus and the accompanying inflammation be of a low-level nature, the quantity of cytokines secreted by the cytotoxic T cells themselves may not suffice, in which case the induction of a CD8 T cell response will be reduced unless additional cytokines are provided by T helper cells. The cytotoxic activity of CD8 T cells is mediated via contact and perforin release (perforin renders the membrane of the target cell permeable resulting in cellular death). CD8 T cells also function in interleukin release (mainly of IFNγ) by which they mediate noncytotoxic effector functions. Perforin-dependent cytolysis is important for the control of noncytopathic viruses, tumors, and transformed cells, but also plays a large role in the control of highly virulent viruses that produce syncytia (e.g., the smallpox virus). Release of noncytolytic effector molecules by CD8 cells, mostly IFNγ, plays a major role in control of cytopathic viruses and intracellular bacteria. Cytolytic effector mechanisms may also contribute to release of intracellular microorganisms and parasites (e.g., tuberculosis) from cells that only express MHC class I.

ANTI-INFECTIOUS IMMUNITY

Theme topicality. The state of the immune system influences on the beginnings of the infection diseases. The desises influence on the humoral and cellular indexes.

Primary objective: According with knowledge about state of the immune system and character of the immune system reaction with different infection diseases to be able treat prevent and diagnose these diseases.

QESTIONS FOR DISCUSSION

1. Anti-infectious immunity: definition, classification. Variety of anti-infection immunity: humoral, cellular (cytotoxic, inflammatory), antitoxic, antibacterial, antiviral, local, general, antifungal, antiprotozoal, protective, nonprotective, sterile, nonsterile.
2. Types of the immune system reactions, which are formed in response to pathogen ingress into the body and the role of immunity in the pathogenesis of infectious diseases.
3. Participation factors of innate immunity in the pathogenesis of infectious diseases (phagocytosis, natural killer, complement system, acute phase proteins: pentraxin, biogenic amines, histamine and seratonin, eicosanoids, prostaglandins, leukotrienes, cytokines).
4. Participation factors of humoral immunity in the pathogenesis of infectious diseases.
5. Participation factors of cellular cytotoxic immunity in the pathogenesis of infectious diseases.
6. Participation factors of cellular immunity in the pathogenesis of the inflammatory type of infectious diseases.
7. Interaction between innate and addoptive factors in anti-infection immunity.
8. Mechanism used by the microorganism for protection against immune system.
9. Components of the agents that modify the immune response (locus of the pathogen, exo- and endotoxins, enzymes, peptidoglican, capsule antigens, imunoglobulinbinding proteins, etc.).
10. Using of humoral and cellular immunity in the diagnosis, treatment and prevention of infectious disease

PROCEDURE OF PRACTICAL SESSION

1. Examine and record in the protocol immunological preparation with designation of the purpose of their use: diagnosis, treatment, prevention.

2. Evaluate the results of PHAT. PHAT was conducted with the serum of patients with whooping cough at 2 and 12 days of onset. Make a conclusion.

PHAT with the serum of patients with whooping cough

	Serum dilution								
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	DC	SC
I	+	+	+	–	−	−	−	−	−
II	+	+	+	+	+	+	−	−	−

Evaluate the results of CFT. CFT was conducted with the paired serum of the patient with syphilis, evaluate results and make a conclusion.

CFT with the serum of patients with whooping cough

	Serum dilution								
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	DC	SC
I	+	+	+	–	−	−	−	−	−
II	+	+	+	+	+	–	−	−	−

Anti-infectious immunity

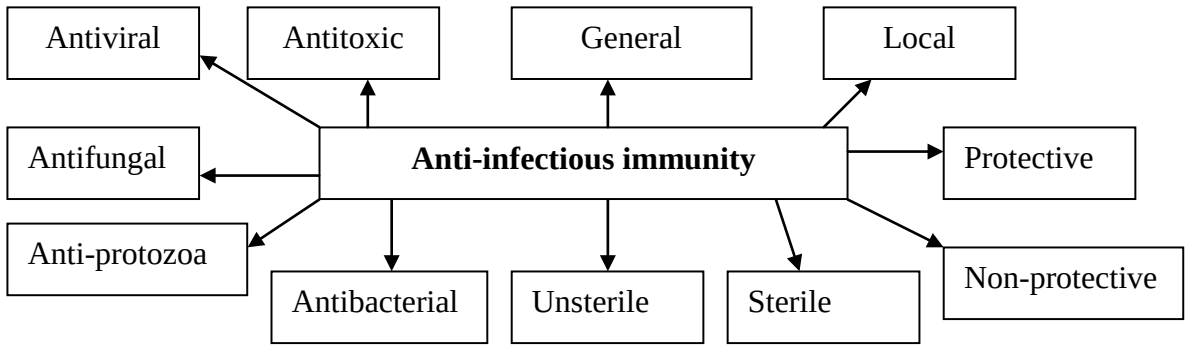
Notion	Definition/explanation

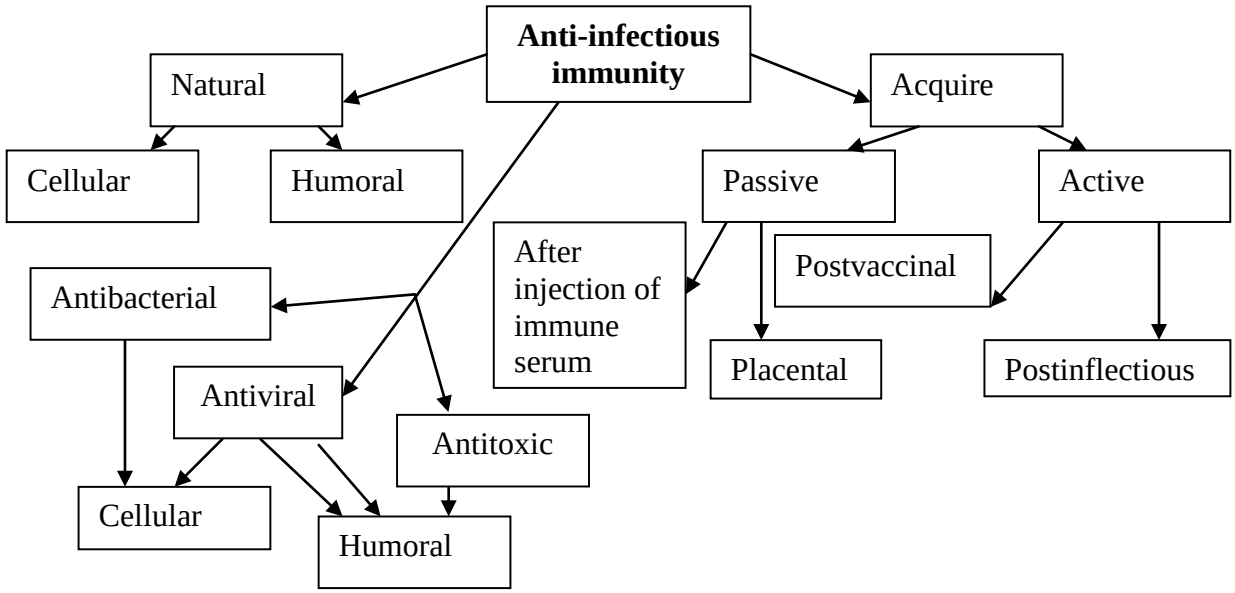
General rules applied to infection protection	**Nonspecific protection** is very important (e.g., toll-like receptors, IFNa/b), and "natural immunity" (meaning not intentionally or specifically induced) represented by natural antibodies, direct complement activation, NK cell and phagocytes, plays a significant role in all infections. However, much remains to be learned about their roles. **Antibodies** represent potent effectors molecules against acute bacterial infections, bacterial toxins, viral reinfections, and in many cases against acute cytopathic primary viral infections (e.g., rabies and influenza). Antibodies are also likely to make a major contribution to the host-parasite balance occurring during chronic parasitic infections. IgA is the most important defence mechanism at mucosal surfaces. **Perforin-dependent** cytotoxicity in CD8 T cells is important for defence against noncytopathic viruses, for the release of chronic intracellular bacteria, and for protection against intracellular stages of certain parasites. **Nonlytic T-cell** responses provide protection in the form of cytokines (very important cytokines include IFNγ and TNFa), which promote the enhanced digestion and destruction of intracellular bacteria and parasites (e.g., listeria, leishmania, etc.), and in some situations enhance immunity against complex viruses (e.g., the smallpox virus). Infectious agents apparently induce cytokines within a matter of hours (for instance IFNγ, IL-12 , and IL-4), and this early cytokine production in turn functions to define the ensuing T cell response as type 1 or type 2. **IgE-mediated defence** is important, along with IgA, in enhancing the elimination of gastrointestinal, pulmonary, and dermal parasites. Although deails of the process are still sketchy, IgE-dependent basophile and eosinophile defence mechanisms have been described for model schistosomal infections
Avoidance strategies of pathogens	**Influence on the complement system.** Some pathogens prevent complement factors from binding to their surfaces: prevention of C4b binding; herpes virus, smallpox virus. **Compartmentalization in non-lymphoid organs.** Viruses can avoid confrontation with the immune defences by restricting their location to peripheral cells and organs located outside of lymphoid tissues: rabies virus; infects neurons. **Modulation and down-regulation of surface antigens.** Infection agents can avoid immune defences by mutating or reducing their expression of T- or B-cell epitopes. Influenza viruses; antigenic shift caused by rearrangement of genetic elements or drift resulting from mutation of haemagglutinin (at the population level). Gonococci; recombination of pili genes. **Interference with phagocytosis and digestion**. *Mycobacterium tuberculosis* uses CR1, CR2, or fibronectin as a receptor for cell entry; it does not oxidative mechanisms in macrophages. Components of bacterial cell walls can impede phagosome-lysosome fusion and are resistant to digestion. **Influence on lymphocytes and immunosuppression.** Direct destruction of lymphocytes, or negative regulation of their function (HIV). **Influence on selection, induction, and deletion of T cells.**

	Negative

Notion	Definition/explanation
	selection of T cells; if viral antigens are present in the thymus responsive T cells will be deleted. **Interference with cytokines, cytokine and chemotaxin receptors (R), etc.** Many viruses produce substances that block or inhibit receptors for the humoral components of the immune system, for instance: IL-1bR, TNFaR, IFNcR; herpesvirus, smallpox virus **Impairment of MHC antigen expression.** Down-regulation of MHC class I and/or class II expression: adenovirus; E19 protein reduces expression of MHC class I on infected cells
Inadequate immune responses to infectious agents	There are a considerable number of inherited immune deficiency diseases that can affect the host response to infection. The reader is referred to other texts for details, but in brief, these defects can result in a variety of immune system changes, including reduced levels of antibody, phagocytic cell alterations, and lack of effector cells. Any of these changes can create a situation where the host is highly susceptible to infections. In some cases, the pathogen ultimately causes immune suppression – an example is infection with HIV, which alters T cell immunity and allows further infection with potential pathogenic pathogens. In other situations, certain bacteria release toxins that function as superantigens, initially stimulating large numbers of T cells to proliferate but, because of the release of cytokines from T cells, ultimately suppressing the immune response and allowing the pathogen to multiply. The pathogen itself may have mechanisms to actively avoid the immune response. For example, several pathogens alter their antigenic structure by mutation to evade the immune protections. Influenza virus undergoes antigenic variation by two mutational mechanisms called antigenic shift and antigenic drift that create new antigenic phenotypes which evade the host's current immunity and allow reinfection with the virus. Several other pathogens have similar evasion strategies – for example, trypanosomes alter their surface glycoproteins and streptococci alter their surface carbohydrate antigens. Examples of other avoidance strategies were discussed earlier, e.g., viral proteins that inhibit the development of an effective immune response. A strategy used by a few pathogens is to become inactive; for example, herpes simplex virus becomes transcriptionally inactive in a state referred to as latency in certain nerve cells after infection and may stay in this state until the immune response declines, whereupon a new cycle of viral replication may be initiated
Antibacterial immune effector mechanisms	**Extracellular bacteria.** Capsules with carbohydrate elements render bacteria more resistant to efficient phagocytosis and digestion (mainly by granulocytes) however, highly repetitive carbohydrate surface antigens induce efficient B cells responses which do not require T help and which are supported in part by lipopolysaccharides (LPS). Pure carbohydrates do not induce T helper. Short-lived IgM responses can control bacteria in the blood effectively, but are usually insufficient in the control of toxins. In such cases, immunoglobulins of the IgG class are more efficient, as a result of their longer half-life and greater facility for diffusing into tissues.

	Intracellular bacteria are controlled by T cells (mainly via T cell secreted IFN-γ and TNF-a which activate macrophages), or in some cases by the release of intracellular bacteria through CD8 T cell mediated cellular destruction





PATOLOGY OF THE IMMUNE SYSTEM

Theme topicality. Disease with the immune system disorders are rather common among the population and tends there occuerence to increase. These are immunodeficiency, allergic, autoimmune, and lymphoproliferative diseases.

Primary objective: to know the pathogenesis of immune diseases and principles of the immunodiagnostics and immunotherapy.

QESTIONS FOR DISCUSSION

1. The disorders types of the immune system functions (immunopathology).
2. Immunodeficiency: definition, classification, clinical manifestations.

3. Principles of the diagnosis and treatment of the immunodeficiency.
4. Allergy (hypersensitivity): definition, types, immunological mechanisms.
5. The principles of the diagnosis and treatment of allergic diseases.
6. Autoimmune (autoaggressive) disease: definition, mechanisms of development.
7. The principles of treatment and prevention of autoimmune diseases.

PROCEDURE OF PRACTICAL SESSION

Study and record in the protocol immunological preparations and mark the aim of their using.

Preparations which cause hypersensitivity:

1. Antitoxic serum: antidiphtheric, antitetanus, against botulism, gas gangrene.
2. Vaccines: against tuberculosis (BCG), brucellosis, anthrax, tularemia, plague.
3. Allergens: tuberculin, brucellin, antraxin, tularin.

Pathology of the immune system

Notion	Definition/explanation
Kinds of the immunopathology	1. Immunodeficiency is immunological deficiency that arise a result of the defects of the immune system or actions damaging factors. 2. Hypersensitivity is a result of distorted high response to specific antigens, allergens, which is called allergy. 3. Autoimmunity arising from the development of immune responses (humoral and cellular) antigens on their body (self-antigen). Developed as a result of violation of self-tolerance mechanisms. 4. Lymphoproliferative processes are a malignant disease in which there is uncontrolled proliferation lymphoid cells
Immune defects	The most important and frequent immune defects are acquired, e.g., iatrogenic (cytostatics, cortisone, irradiation, etc.), age-induced, or the result of viral infections (above all HIV). Congenital defects are rare; examples include Bruton's X-chromosome-linked B-cell defect, thymus hypoplasia (Di George), and combined T- and B-cell deficiency resulting from MHC defects (bare lymphocyte syndrome) or from enzyme defects (adenosine deaminase [ADA] deficiency or purine nucleoside phosphorylase [PNP] deficiency). These defects can also be repaired by reconstitution (thymus transplants), or in some cases through the use of stem cells (gene therapy; one of the very first successful gene therapies was the treatment of ADA deficiency). More frequent congenital defects involve selective deficiencies, for example a relative-to-absolute IgA deficiency, normally being more prominent in infants than later in life. Children with such deficiencies are

Notion	Definition/explanation
	more susceptible to infection with *Haemophilus influenzae*, pneumococci, and meningococci. General consequences of immune defects include recurring and unusual infections, eczemas, and diarrhoea
Autoimmune pathology or immunopathology	Some clinically important auto-antibodies are directed against hormone receptors, for example thyrotoxicosis in Basedow's disease is caused by auto-antibodies that stimulate the TSH receptor, and myasthenia gravis is caused by blockage of the acetylcholine receptor by specific auto-antibodies. Other antibody-induced diseases mediated by antibodies, directed against hormones and other cellular self antigens, include

	Hashimoto thyroiditis (induced by anti-thyroglobulin and anti-mitochondrial auto-antibodies), pernicious anaemia (anti-intrinsic factor), pemphigus vulgaris (anti-desmosome), Guillain-Barre syndrome (ascending paralysis caused by specific myelin auto-antibodies), and scleroderma (involving anti-collagen antibodies). Other immuno-pathologies involving auto-antibodies include transplant rejection as a result of endothelial damage (especially in xenogeneic transplants), and tumor rejection caused by antibodies against tumor-associated antigens present on neoplastic cells (especially relevant for lympho-haematopoietic tumors). However, in general the detection of auto-antibodies does not necessarily correlate with evidence of pathological changes or processes. In fact, our detection methods often measure low-avidity auto-antibodies that may have no direct disease-causing effects. Exactly how autoantibody responses are induced remains to be clarified. As explained earlier (in the discussion of immunological tolerance) such IgG responses cannot be induced without T help. Thus, intensive research is currently focused on those mechanisms by which T cell help for auto-reactive B cells is regulated
Atopy	It is the inherited predisposition to develop first (immediate) type of the hypersensitivity on the introduction the specific antigens-allergens. It is stipulated by increased IgE production
Principles of the allergic diseases treatment	1. Exemption from body contact with allergens. 2. Immunosuppressive therapy: depression the humoral or cellular chain of the allergy pathogenesis. 3. Bloc of the immune reactions mediators by antihistamine preparation. 4. Anti inflammation preparations (corticosteroids, etc.). 5. Specific immunotherapy: immunization a specific allergens to synthesis of the protective IgG, which compete with IgE
Prevention of anaphylactic shock	When you inject all serum preparation the sensitive organism to introduced serum is detected. The children have the serotherapy particularly frequently. Reintroduction of the serum may lead to increased sensitivity of the children organism to this drug, so you need to make skin test. This is introduced intracutaneously 0.1 ml of the serum in 1:100 dilutions. If after 30 minutes blister and hyperaemia does not form, you can inject the serum. Therapeutic dose of serum is introduced separately by Bezredko's method to prevent the development of the anaphylactic shock. First, we introduce 1/3 dose and after 2 - 3 hours – another part of the sera
The types of immune	- normergy - adequate protective immune response;

Notion	Definition/explanation
reactions	- hyperergy - distortion, increased immune response (allergy); - hypoergy - reduced immune response; anergy - no immune reactions
The principles of the autoimmune diseases treatment	1. Immunosuppressive therapy is used for inhibition of humoral or cellular chain pathogenesis. 2. Election exclusion "forbidden" clones sensitized lymphocytes or autoantibody (haemosorption, plasmophoresis). 3. Anti-inflammation drugs are used (corticosteroids, salicylic acid

	preparations). 4. It is blocking of the immune reactions mediators by using of the antihistamin and other drugs. 5. Immune-correction of the deficiency or functional defect of the T-suppressors
Classification of the immunodeficiency	**Primary** immunodeficiency is a condition that is result a genetic or developmental defect in the immune system. In such condition, the defect is present in birth, although it may not manifest itself until later in life. **Secondary or acquired** immunodeficiency is the loss of the functions and result of exposure to various agents. The immunodeficiency may be classified by the cells types involved and may affect either the lymphoid- or myeloid- cells lineage or both
Principles of the immunodeficiency treatment	Although there are no cures for immunodeficiency there are several treatment possibilities: - replacement of a missing proteins; - replacement of a missing cells type or liners; - replacement of a missing or defective genes

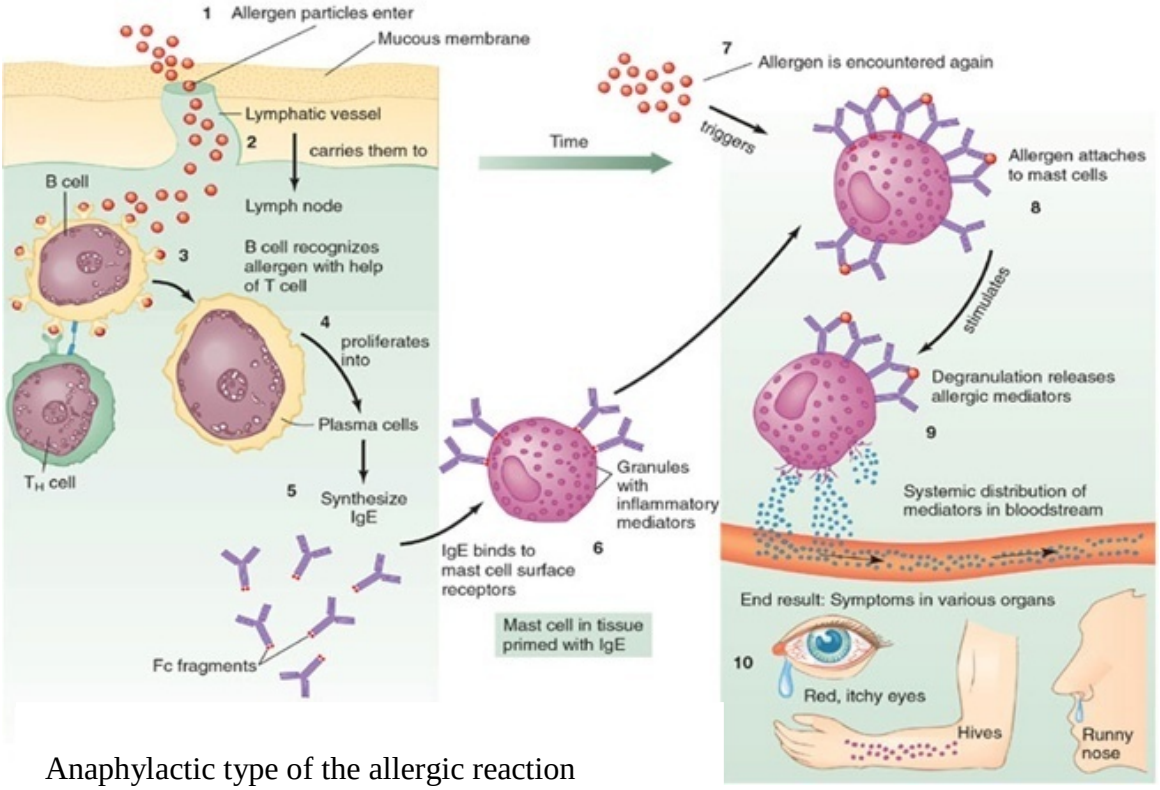
Types of the allergic reactions

Type number	Name and date of the clinical manifestations	Immune-pathological mechanism	Example of clinical manifestations
I	Anaphylactic – up to 30 minutes	In response to the antigen Ig E synthesized that sorbs on the mast cells and basophiles. After that AG connect with these AB and forms a complex AG + AB and cells produce mediators like histamine	Atopic bronchial asthma, rhinitis, anaphylactic shock (medicinal, etc.)
II	Cytotoxic 5–12 h	It is synthesizing the IgG and IgM against antigens of the cell membranes that have changed and are self-antigen. Cytolysis occurs in the reaction AG + AB through activation of the complement	Medical allergies, haemolytic anaemia, thrombocytopenia, auto-sensibilization to the antigens of the thyroid gland, kidney basal membrane, etc
III	Immunocomplex 3 – 8 h	The complex Ag + AB forms as a result of the simultaneous their circulation in the blood. These complexes sediment on the wall of the vessels and connect with complement. As a result the vessel wall are demaged	Serum disease, autoimmune diseases (collagenoses). Complications of the infectious diseases
IV	Cellular (DTH) 24 –48 h	In this type we see the accumulation of the sensitized TH1 lymphocytes. As a result there is the reaction between antigen and TDTH and synthesis of the	Allergy in infectious diseases, contact allergy (medication, etc.)

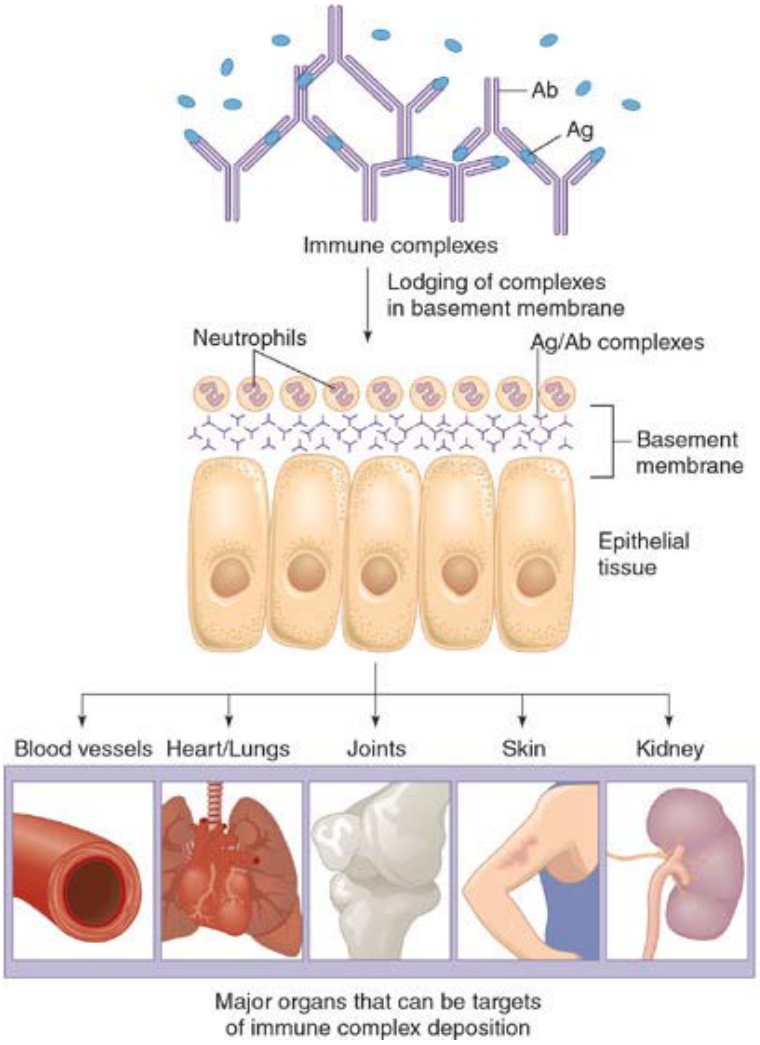
		cytokines (INFα, IL2), which activate macrophages to produce proinflammatory cytokines (IL1, TNFγ, IL-6), that cause the inflammation	

Mechanisms of autoantibody induction

Possible mechanisms	Autoimmune pathology or immunopathology
Polyclonal B-cell activation	Lipopolysaccharides, viruses, chronic parasitic infection
Molecular mimicry (overall very rare)	Anti-tat (HTLV-1), anti-H. pylori, or anti-streptococcus crossreacting with self-antigens
Exposure of hidden auto-antigens	Cytopathic effects of infectious agents
Adjuvant effects	In the presence of granuloma formation and chronic inflammatory reactions lymphoid tissue may form in peripheral organs (e.g., during Hashimoto's thyroiditis)
Breakdown of tolerance	Due to coupling of T helper epitopes to autoantigens, possible in connection with virus infections of cells



Anaphylactic type of the allergic reaction



III (Immunocomplex) type of the allergic reaction

EVALUATION OF THE IMMUNE STATUS. PRINCIPLES OF THE IMMUNE SYSTEM FUNCTIONING

Theme topicality. Definition of the immune status of patients and healthy people should be performed under suspicion in presence of immunodeficiency, as well as those who will live and work in extreme conditions.

The immune system as other systems of the body has its own peculiarities of functioning. Doctor should to know and take into account these principles in his professional activity.

Primary objective: to be able to evaluate the immune status and recognize human immunodeficiency. To assimilate the principles of the immune system and features of the anti-infectious immunity.

QESTIONS FOR DISCUSSION

1. Immune status of the body: definition, principles and indications for research.
2. Purposes and methods of the determining the state of cellular immunity.

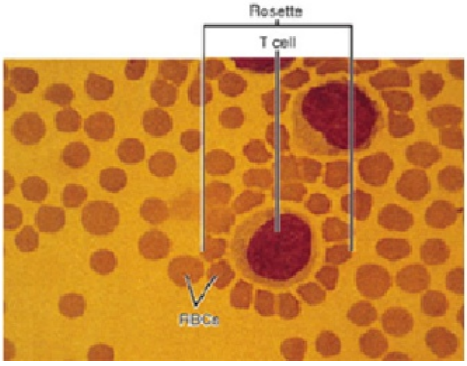
3. Two-stage principle of the immune status estimation.

First-stage tests (tentative):
- clinical analysis of peripheral blood. Relative and absolute number of lymphocytes in the blood;
- determination of the number of the T-and B-lymphocytes in the blood;
- identification of major classes of immunoglobulin (M, G, A) in serum;
- identification of the phagocytic activity of the leukocytes.

Second-stage tests (analytical):
- identification of the subpopulations of the regulatory T-lymphocytes (T-helpers, suppressor cells);
- identification of the spontaneous migration of the leukocytes and leukocytes migration inhibition test with the use of PHA;
- skin allergic test with tuberculin and other allergens to which there are sensibilization in most populations;
- study of the proliferative activity of T-lymphocytes in response to mitogens, antigens in blast-transformation test;
- determination of the dynamics of major cytokines that regulate cellular and humoral immune response (INF-γ, TNF-α, IL-2 and IL-4, 5, 6, 10) and modulate inflammation.

4. Principles of the immune system functioning.
5. The aims and methods of the determining the state of humoral and cellular anti-infectious immunity.
6. Components of the pathogens (bacteria) that modify immune response (localization of the causative agents, exotoxin and endotoxin, enzymes, peptidoglican, capsule, immunoglobulin-binding proteins, antigens, etc.).
7. Mechanisms of the microorganism's protection from the immunity factors.

PROCEDURE OF PRACTICAL SESSION



Rosette forming test

Study the determination of the T-lymphocytes (CD3) total number in stained smears from the peripheral blood by spontaneous rosette test

Determination of T-lymphocytes (CD3) total number in human blood is one of the leading indicators of the cellular immunity state. The principle of the spontaneous rosette test is connected of the human T-lymphocytes by CD3 receptor with sheep blood erythrocytes.

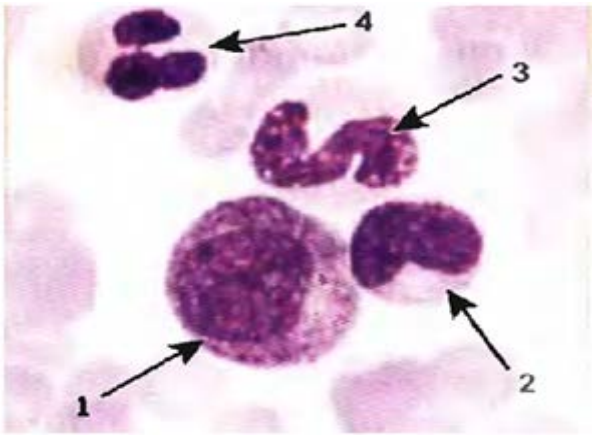
The reaction consists of the following stages:
1. Preparation of the sheep erythrocytes suspension in the growth medium 199.
2. Preliminary separation of the lymphocytes mixture from the blood.
3. Mixture in the centrifugal test-tube equal volumes (0.5 ml) of the lymphocytes and erythrocytes suspension. Incubate the mixture at 37 °C (10–15 min), centrifuge at 1000

revolutions 1 minute and gently place the tube in a refrigerator at 4 °C for 1–2 hours or leave overnight.

4. Sediment gently mixed by rotating the tube around its axis in the hands. Put the drop of the suspension in the special camera and caunt under the microscope 100–200 lymphocytes, which are attached and not attached the erythrocytes.

Rosette-forming cell is considered the lymphocyte that joined three or more erythrocytes. Put the mixture to the microscope fatless slide for prepare the cell smear, dry it at room temperature, then fix by glutar aldehydes, washed with water and stain it by Romanovsky-Gimze. The lymphocytes are purple, erythrocytes – pink in the stain condition. There are 55–80% (800–2000) rosette-forming lymphocytes in the adult blood in the normal. You must determine the relative (%) and absolute (1 ml) quantity of the rosette-forming lymphocytes. You must find the rosette-forming lymphocytes in the smear and sketch in protocol.

2. Study in the stained smear of the blood the blast-transformation of lymphocytes, stimulated by nonspecific mitogens – phytohaemagglutinin (PHA).



Blast-transformation of lymphocytes

Reaction of lymphocytes blastic transformation (RLBT) is studied when the function of lymphocytes is estimated. Blast-transformation is the migration of the lymphocytes from a quiet state (number 3, 4) in blast form (number 1, 2), that are capable to proliferate and differentiate further.

This process is accompanied by morphological changes in lymphocytes: their size increase, number of mitochondria, ribosomes. Blast is a large (20-24 mkm in diameter and more than 40 microns), rounded cell. Nucleus of this cell occupies most of the cytoplasm. Cytoplasm of the blast is granular, basophile with light zone around the nucleus. There is intensive synthesis of the protein, RNA and DNA in the lymphocytes during their transformation in the blast. The process finishes the mitotic division of the cells. Specific and nonspecific stimulants (mitogens) may cause the RLBT. Nonspecific phytogenic mitogen is phytohaemaagglutinin (PHA). Specific mitogen is soluble and corpuscular antigens that act with T-lymphocytes. Blast-transformation is studied in vitro in the lymphocyte culture in the sterile conditions in the medium 199. The optimal concentration of the lymphocytes is 10^6 in 1 ml medium. The maximal concentration of the lymphocytes blasts is in culture within 2–4 days after mitogen inoculation. 70–80% of peripheral human blood lymphocytes are transformed into blasts under the influence of PHA. RLBT reflects the functional activity of T-cells. Reaction is determined by radiometric method if there are the equipment and reagents. Micromodification of the RLBT with radioactive isotopes is widely used. Blastic transformation is estimated by the detection of the level of the labeled tritium timidin ($_3$H-timidin) inclusion in the DNA of lymphocytes. Results reflect the number of

pulses per minute with an automatic scintillation counter. RBTL can be assessed by morphologically. It is direct counting the blasts under the microscope in stained smears. You must find the blasts during the microscopic examination of the smears and sketch them in the protocol.

Study the leukocytes migration inhibition test in capillaries.

Leukocytes migration inhibition reaction is made for functional evaluation of sensibilized T-lymphocytes, which together with the specific allergen-coated antigens produce cytokine, which inhibits migration of leukocytes in foci of inflammation. This cytokine (haemokin) is called migration inhibitory factor (MIF). Migration square in the study and control is measured under low magnification of the microscope, then the index of migration is calculated to the formula MI = S/PC, where S - area of migration in experiments, PC - area of migration in control (without Ag).

Study the situation task and record conclusion in the protocols.

Study the immunogrames.

Example 1. Define the presence of the congenital immunodeficiency in the man of 55, who during two years had three investigations for the content the major immunoglobulin in peripheral blood. Results of the study:

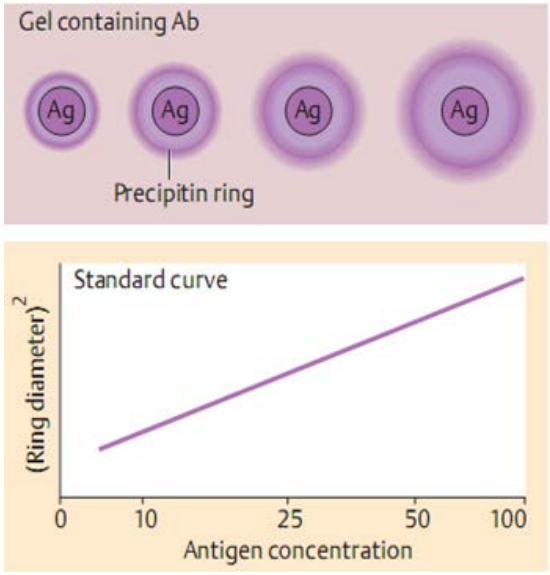
Man of 55	Ig (g / l)		
	A	M	G
A	0	2.7	30.3
b	0	1.9	39.0
c	0	2.1	28.5

Evaluation of the immune status. Principles of the immune system functioning

Notion	**Definition/explanation**
Immune status	It is the human body clinical state, quantitative and functional state of the humoral and cellular immunity factors
The indications for the investigation of the immune status	The indications for investigation of the immune status are propensity to chronic purulent-septic, cancer, and other diseases, when immunodeficiency is suspected, healthy individuals who will work in extreme (harmful) conditions
The principles of the immune status assessment	1. Two-stage principle. 2. Link principle study of the immune system (phagocytosis, cellular, and humoral link), depending on the clinical manifestation of the immunodeficiency
The principles of the immune system functioning	1. The principle of local effector action of the immunity factors. 2. The principle of cascade action (chain reaction). 3. The principle of the speed and balance of the immune reactions activation and inhibition

Notion	**Definition/explanation**

	4.The principle of the efficiency increasing with the experience acquisition (immunological memory after contact with an alien). 5. The principle of the redundancy of the immune mechanisms with the possibility of compensation (overlapping) the functions by components of the immune system
The aims and methods of the anti-infectious humoral and cellular immunity research	1. Diagnosis of infectious diseases and complications 2. Determination of the treatment effectiveness. 3. Predection of the infectious diseases course and definition of the complications. 4. Determination of the vaccinations effectiveness. For these aims one should use: - serological method (determination of specific antibodies); - determination of the type and serotype of pathogens in their antigenic properties at the end of the bacteriological method
Immunogram	Advanced clinical analysis of blood with immunological parameters (number of T and B lymphocytes, etc.), which indicate the status of immunity
Determination of the T and B lymphocytes number in the blood	Determination of the T and B lymphocytes number in the blood is made by means of: - rosette-forming test; - IFT with monoclonal antibodies; - fluorescence-activated cell sorter (FACS)
Identification of the major classes of immunoglobulin (M, G, A) in serum	Identification of major classes of immunoglobulin is made by means of: - radial immunodiffusion according to Mancini; - ELISA
Identification of the phagocytic activity of the leukocytes	It is made by determination of: - phagocytic numerous; - phagocytic index
Identification of the subpopulations of the T lymphocytes	It is made by means of: - IFT with monoclonal antibodies; - fluorescence-activated cell sorter (FACS)
Determination of the functional activities of leukocytes	Identification of the spontaneous migration and migration inhibition test with the use of PHA are made for functional evaluation of sensibilizated T lymphocytes, which together with the specific allergen-coated (antigens) produce cytokine (MIF), which inhibits migration of leukocytes in foci of inflammation
Study of the proliferate activity of T-lymphocytes in response to mitogens, antigens in blast-transformation test	Blastic transformation is the migration of lymphocytes from a quiet state in blast form that are capable to proliferate and differentiate further. Specific and nonspecific stimulants (mitogens) may cause the RLBT. Nonspecific phytogenic mitogen is phytohaemagglutinin (PHA). Specific mitogen is soluble and corpuscular antigens that act with T lymphocytes. 70–80% of peripheral human blood lymphocytes are transformed into blasts under the influence of PHA. RLBT reflects the functional activity of T cells
Test used for determination of the cytocines dynamics	ELISA



Radial immunodiffusion according to Mancini

Quantitative assay of an antigen uses specific anti-serum which is mixed with agar and poured into a plate. The antigen is then diluted to different concentrations, and pipetted into wells that have been previously punched into the plate. Antigen-antibody complexes precipitate in the form of a ring around the well, the diameter of which is proportional to the antigen concentration. The result is a standard curve from which unknown test antigens can be quantified. Analogously, antibodies can also be quantified by mixing antigens in the gel.

Immune status. Immunodeficiency diseases and immune correction

I. INDEPENDENT STUDY PROGRAM

1. Indication for an assessment of immune status.

2. 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.

3. 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.

4. 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.

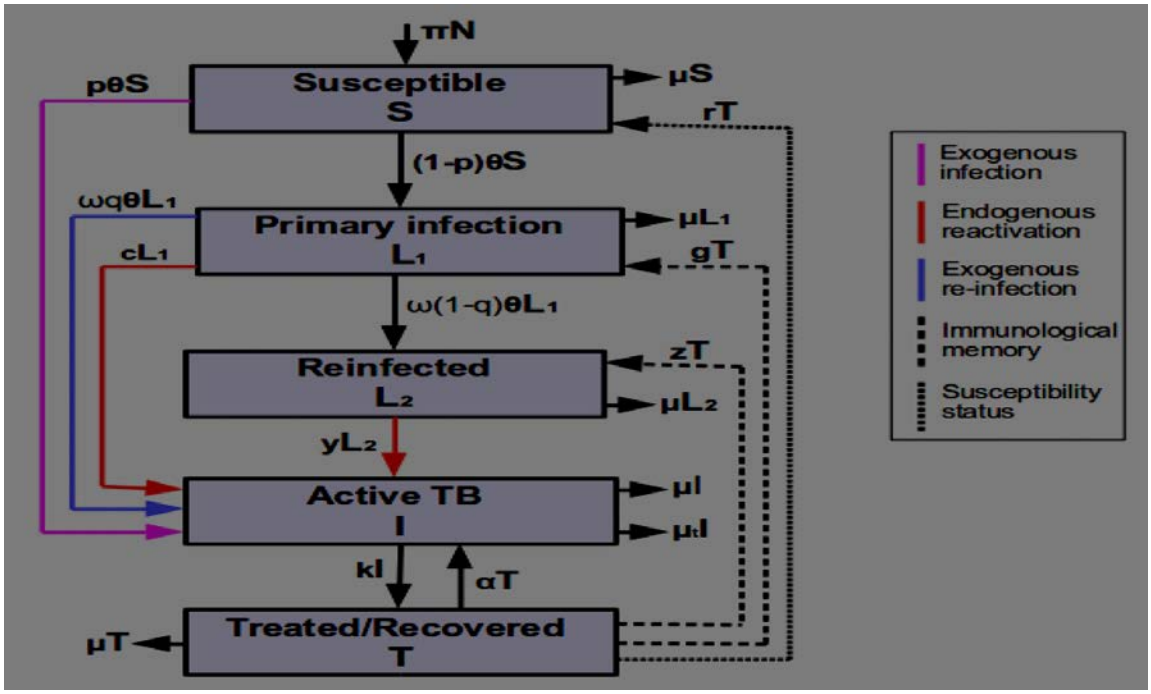
6. Tumour immunology.

7. 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;

Table 1

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

The immune status of the person.

Characteristics of person

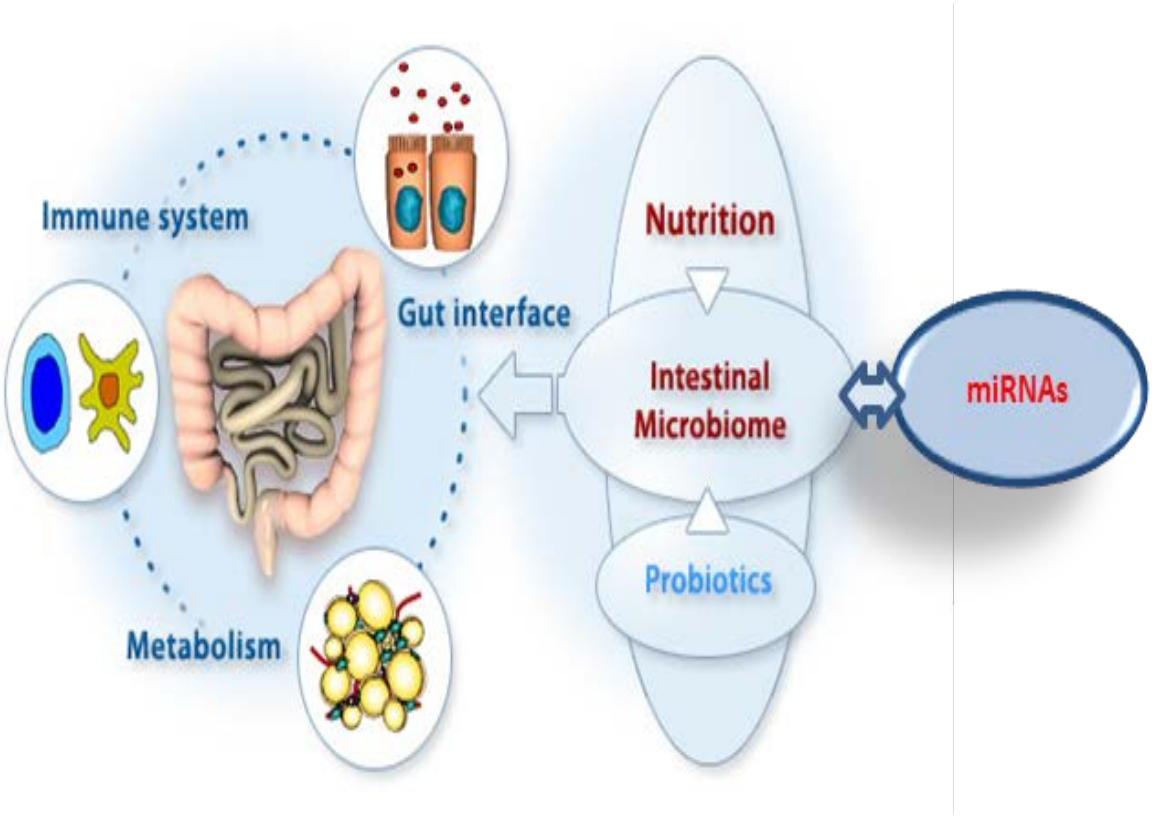
Personal characteristics may affect illness, organization and analysis of data by “person” may use

- Inherent characteristics of people (for example, age, sex, rase).
- Biologic characteristics (immune status).
- Acquired characteristics (marital status).
- Activities (occupation, leisure activities use of medications /tobacco/ drugs), or the conditions under which they live (socioeconomic status, access to medical care).
- Age and sex are included in almost all data sets and are the two most commonly analyzed “person” characteristics.
- However, depending on the disease and the data available, analyses of other person variables are usually necessary.
- Usually epidemiologists begin the analysis of person data be looking at each variable separately. Sometimes, two variables such as age and sex can be examined simultaneously.
- Person data are usually displayed in tables or graphs.

The state of functional activity of the human immune system as a whole is vital to the body and is denoted by the term "immune status".

Immune status are indicators of nonspecific and specific defenses, which determines the immune status of the person.

Fully system protective factors developed for 15-16 years. As the body ages, the immune system weakens, there is a risk of immunodeficiency, autoimm. States, malignant tumors, etc.



Types of disorders of the immune system:

* *Failure of the immune system* or immune deficiency. Reduced activity of the immune system, developing in the reduced number of components of the immune system or their lack of functional activity.

* *Hyper-reactive immune system.* Excessive activity of the immune system, which can lead to serious over caused her illness.

* *Autoimmune reactions*. The immune system attacks its own tissues. There is the breakdown of immunological tolerance to antigens own tissues.

The functional state of the immune system is determined by a set of specific and nonspecific indicators of the work as individual units, and the system as a whole.

These indicators can be measured and quantified, as is known for their level in the normal functioning of the immune system and can be observed deviations for violations of its work.

The indicators characterizing the state of specificity. link the immune system:

- the level of immunoglobulins of all classes in the blood;
- the number and functional activity of T - and b-lymphocytes and their subpopulations;
- the severity of cellular and humoral immunity to the introduction of antigens;
- the reaction GST and skin reactions;
- system state immunocytokines;
- the activity of the immune phagocytosis and other.

Condition factors determining natural resistance, take into account by determining the content of macrophages and their phagocytic capacity, the functioning of normal cells, blood complement, interferon, some enzymes (lysozyme) and inhibitors.

The set of indicators specific and nonspecific nature determines the immune status of the organism, i.e. the immune status.

The immune status of the body is in dynamic equilibrium, as it always act environmental factors (climatic, socio-biological, environmental) and endogenous factors affecting physiological and biochemical processes in the body.

Therefore, the immune status is primarily determined by the physiological state of the organism as a whole.

Disruption of normal immune status, defective functioning of the immune system called immunodeficiencies.

Immunodeficiency is divided into primary (congenital) and secondary (acquired).

Primary (congenital) immunodeficiency - when congenital genetic defects anatomic and functional character. This human diseases that manifest in early childhood and often lead to death, because the body cannot provide enough resistance to various infectious agents.

Violation occurs the production of stem cells and limit their ability to differentiate to T - and b-lymphocytes. The body is not able to produce antibodies.

Secondary (acquired) immunodeficiency - after previous infections (especially viral), tumors, metabolic disorders, severe trauma, during aging.
As both can affect or is a shortage of T - or b-system of immunity, i.e., failure of cellular or humoral, or to be combined (the failure of T - and b-system). An example of a congenital deficiency In the system is agamma-globulinemia (the inability to produce γ-globulins), and failure of the T-system - hypoplasia of the thymus gland (syndrome Di George).

Meet congenital defects of phagocytic system, complement and other parts of the immune system
Secondary immunodeficiencies are developing in many bacterial and viral infections, tumors, exposure to body substances that have suppressive effect (some drugs, antibiotics), the impact of occupational hazards, etc.

Primary and secondary immunodeficiencies are caused by many diseases, most often to the emergence of infections (pneumonia, gastrointestinal disease, purulent and inflammatory diseases of the skin, joints, nervous, urogenital system, and so on), and neoplastic processes.

A good example are secondary infections affecting the body for HIV infection.

As the immune status depends on two large interconnected systems - humoral and cellular immunity, it is their condition reflects a number of immunological tests in the immunological.

Most often it is the analysis of venous blood, but sometimes there is a need to explore the local immunity of the mucous membranes or to conduct skin tests with allergens.

To evaluate **humoral immune status** help methods for detection and

enumeration of b-lymphocytes in the blood (in absolute numbers and percentage). The functional activity of b-lymphocytes detected, determining synthesized them immunoglobulins. For this blood to measure the total concentration of immunoglobulins and the number of immunoglobulins of different classes (IgA, IgM, IgG), determine the presence of antibodies to common bacteria and viruses, as well as the level of autoantibodies.

The definition of the complement system is important in the diagnosis of congenital immunodeficiencies, in other cases, for example, in autoimmune diseases, we need only the data on the C3 and C4 components of complement. (Activation To involve at, alternately formed all 9 components from C1 to C9).

Cellular immune status in norm particularly effective against fungi, parasites, intracellular infections, cancer cells and evaluated the methods in which we can determine the number of T-lymphocytes in the blood and their functional activity.

Be sure to determine the number of different subpopulations of T-lymphocytes (suppressor, helper), their functional activity, and cytotoxic activity of K - and CS - killer cells.

Evaluate the products the peripheral blood lymphocytes of humoral mediators of cellular immunity (interferons, interleukins, cytokines).
All this is useful for the study of immune deficiencies that are associated with chronic infections and to monitor the use of immunostimulatory therapy.
Determine the phagocytic index (phagocytic activity) - the percentage of phagocytes from among the regarded cell. Phagocytic number (phagocytic index) is the average number of microbes, absorbed one active phagocytosis..

Determination of the immune status of the person.

There are screening (from the English. screening - "selection sort") tests for assessing the immune status that allow you to quickly evaluate the basic performance of the immune system.

The standard screening test includes:

* Counting the absolute number of leukocytes, neutrophils, lymphocytes and platelets.
* Determination of the concentration of serum immunoglobulins of different classes (IgG, IgA, and IgM).
* Determination of the hemolytic activity of the complement system.
* Conduct skin tests of delayed-type hypersensitivity (GST).

A more detailed study of the immune status includes the study of the number and functional activity of the cellular and humoral immune system:
* Study of the phagocytic function of macrophages.

* The study of the complement system (the content in the serum of complement, lysozyme, interferon).
* Research the T-system of immunity (cellular immunity).
* Research In-immunity (humoral immunity).

A more detailed study of the immune status is carried out in several stages. First, make tentative studies to identify the major defects of the immune system (level 1), and then may need a more detailed study (level 2), based on data from previous studies.

Indicators can be extended or not fully defined. Specific immunity against infectious diseases must be determined.

When abnormalities in the immune system of the patient can assign immunotropic drugs, such as Immunostimulants, immunomodulators or immunosuppressants.

Can also be conducted substitution therapy using immune sera, immunoglobulins, leukocyte mass or preparations of interferons.

Immunodeficiency underlying many diseases and clinical manifestations, can be treated and cured. For this purpose apply immunostimulants and immunosuppressive therapy.

IMMUNODEFICIENCIES.

Failures of the immune response can compromise the ability of the human body to resist infection.

Such failures may be due to an inadequate or an inappropriate immune response.

If an individual has an inadequate immune response **(immunodeficiency),** he or she will not be protected against many infectious diseases.

Individuals with immunodeficiencies are subject to numerous infections with opportunistic pathogens (Table 2).

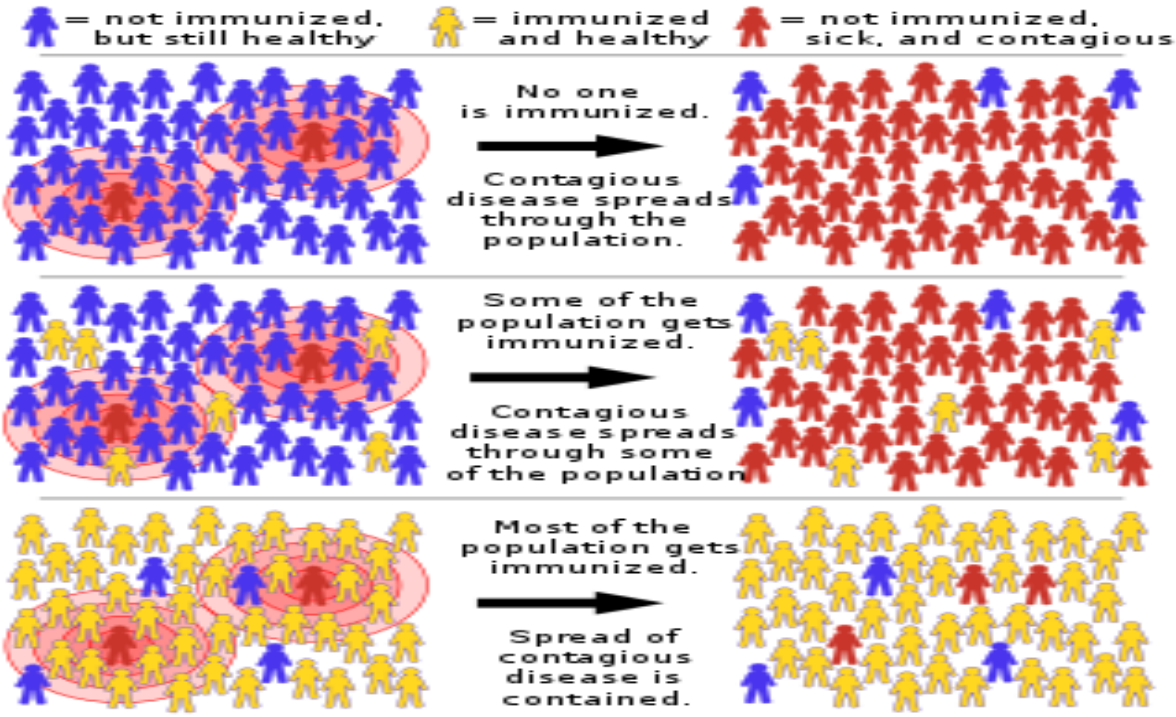
Immunodeficiencies may be congenital, that is, the result of an inherited genetic abnormality or acquired from external causes at some time during the life of the individual.

Table 2

Common Infections in Immunocompromised Individuals

Deficiency	Infecting agent
Damaged tissues (burns, wounds, trauma)	*Aspergillus* species, *Candida* species, *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes*
T lymphocytes	Cytomegaloviruses, herpes simplex viruses, varicella-zoster virus, *Listeria monocytogenes, Mycobactenum* species, *Nocardia* species, *Aspergillus* species, *Candida* species, *Cryptococcus neoformans, Histoplasma capsu- latum, Pneumocystts carinii, Strongyloides stercoralis*
B lymphocytes	*Staphylococcus aureus. Streptococcus* species, *Haemophilus influenzae, Neisseria meningitidis, Escherichia coil, Giardia lamblia, Pneumocystis carinii*

Severe combined immunodeficiency	Same infecting agents for T and B lymphocyte deficiencies
Phagocytic cells (PMNs and macrophages)	*Aspergillus* species, *Candida* species, *Nocardia* species, *Staphylococcus aureus. Streptococcus pyogenes, Haemophilus influenzae, Escherichia coli, Klebsiella* species, and *Pseudomonas aeruginosa*
Complement	*Staphylococcus aureus. Streptococcus pneumoniae, Pseudomonas, Proteus, Neisseria* species



SEVERE COMBINED IMMUNODEFICIENCY.

The most devastating type of congenital immunodeficiency is **severe combined immunodeficiency** (SCID).

Individuals with severe combined deficiency have neither functional B nor T lymphocytes. Such individuals are incapable of any immunological response.

Any exposure of such individuals to microorganisms can result in the unchecked growth of the microorganisms within the body.

This results in certain death. Individuals suffering from severe combined deficiency can be kept alive m sterile environments.

They must be protected from any exposure to microorganisms. In a well-publicized case, a boy named David was kept alive in a sterile bubble chamber for 14 years.

Everything entering the chamber—air, water, food—was sterilized. As long as he was not exposed to microorganisms, he was able to survive.

Tragically, he died as a result of an attempt to cure his immunodeficiency. He was given a marrow graft from a sibling with compatible bone marrow in an attempt to establish functional lymphocytes in his body.

He developed an adverse reaction that proved fatal.

In other cases, such bone marrow transplants have been effective, including some performed within weeks of the unsuccessful treatment of David.

A new treatment for some cases of SCID is the administration of the enzyme adenosine deaminase (ADA) Accumulation of adenosine compounds is toxic to lymphocytes and ADA is needed to prevent toxicity. In the absence of ADA, B and T lymphocytes die. Approximately 35% of the cases of SCID are due to ADA deficiency.

Administering ADA can be therapeutic as long as it is not detected as a foreign antigen.

To block its recognition as an antigen that would trigger an adverse immune reaction, the ADA is chemically linked to polyethylene glycol (PEG). PEG coats the ADA and blocks its recognition as an antigen. PEG-ADA treatment is being used effectively to treat some cases of SCID.

The inability to produce ADA in an individual with SCID is due to a defective gene. Gene therapy is also being tried in an attempt to cure this condition (FIG. 1).

Cells can be obtained from a patient and a functional gene for ADA production inserted into the cells by genetic engineering.

The recombinant cells can then be introduced into the patient.

Early clinical trials have shown significant improvements in the immune responses of children treated with this gene therapy.

DiGEORGE SYNDROME.

DiGeorge syndrome results from a failure of the thymus to develop correctly. It is probably caused by *as* abnormal fetal development that interferes with the proper formation of the thymus.

T lymphocytes in individuals with this disease do not become properly differentiated.

Signs and symptoms of this disease often are apparent at birth.

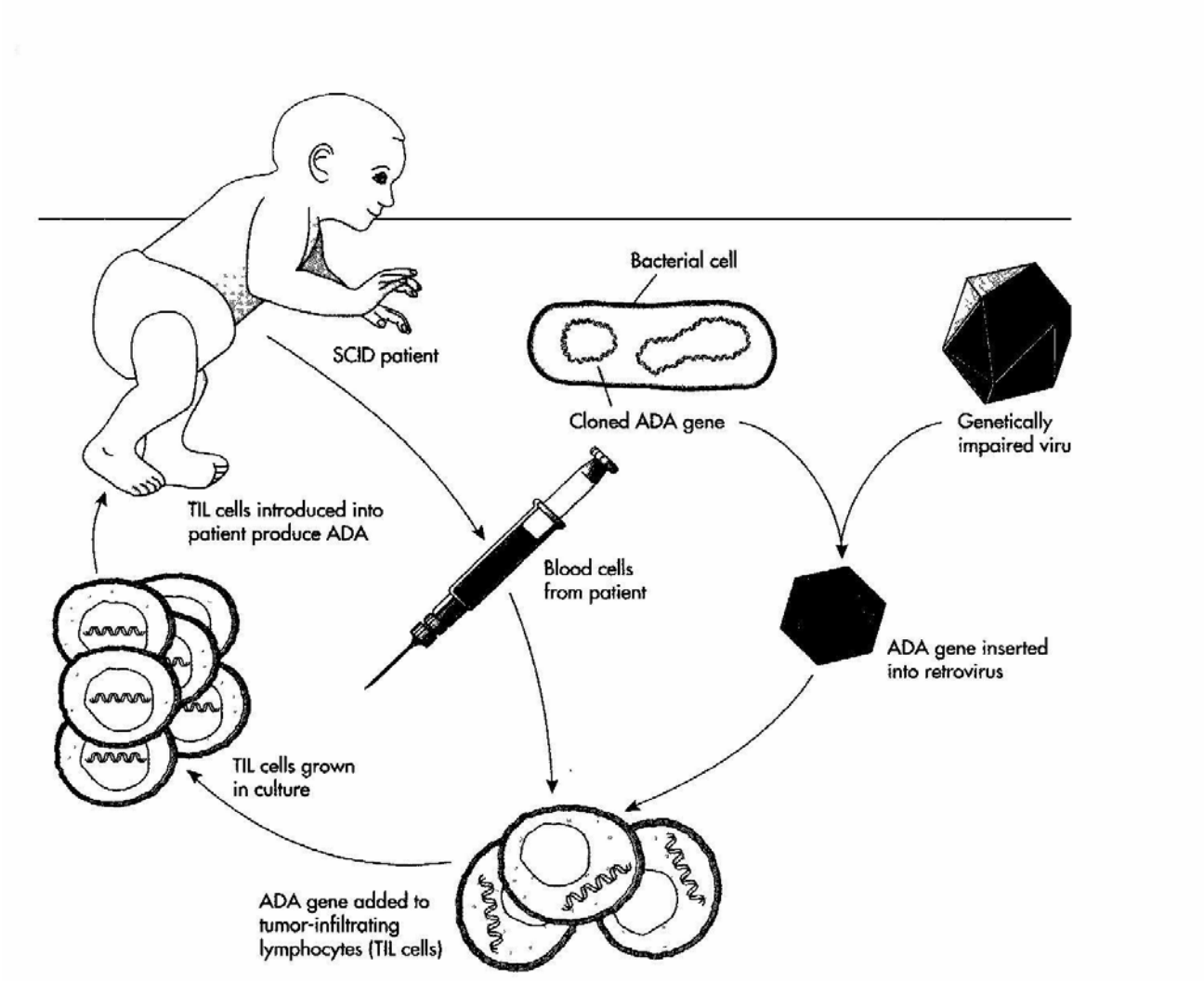
They include deformities such as low-set ears, fish-shaped mouth, undersized jaw, and wide-set eyes Elevated serum phosphate and low serum calcium also are characteristic of DiGeorge syndrome.

A low phosphate diet and calcium supplements are used to achieve acceptable levels of phosphate and calcium in the blood.

Individuals suffering from this condition do not exhibit cell-mediated immunity.

Therefore they are prone to viral and other intracellular infections.

Avoidance of infecting agents is important in the management of patients with DiGeorge syndrome.



PEG-ADA gene therapy can be used in the treatment of SCID

Bruton congenital agammaglobulinemia.

Bruton congenital agammaglobulmemia results in the failure of B cells to differentiate and produce antibodies Individuals with Bruton disease have a normal cell-mediated res-ponse.

This immunodeficiency disease affects only males Boys with Bruton agammaglobulmemia are particularly subject to bacterial infections, including

those by pyrogenic (fever-inducing) bacteria such as *Staphylococcus aureus, Streptococcus pyogenes, S pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae.*

The treatment of this disease involves the repeated administration of pooled gamma globulin to maintain adequate levels of antibody in the circulatory system.

Late-onset hypogammaglobulinemia.

The most common form of immunodeficiency is known as late-onset hypogammaglobulinemia. In this condition, there is a deficiency of circulating B cells and or B cells with IgG surface receptors.

Such individuals are unable to respond adequately to antigen through the normal differentiation of B cell into antibody-secreting plasma cells.

Other immunodefficiencies may affect the synthesis of specific class of antibodies.

For example, some individuals exhibit IgA deficiencies, producing depressed levels of IgA antibodies.

Such individuals are prone to infections of the respiratory tract and body surfaces normally protected by mucosal cells that secrete IgA.

COMPLEMENT AND CELLULAR DEFICIENCIES.

Immunodeficiencies may also result from inadequate functioning of monocytes, neutrophils, and macrophages.

Phagocytic cells lacking enzymes that produce hydrogen peroxide and other antimicrobial forms of oxygen do not have proper lysosomal functions that kill bacteria.

Pathogenic bacteria are able to multiply within such metabolically deficient phagocytes.

Antibiotics can be used to protect individuals who are deficient in both complement and active phagocytic cells against invading pathogenic bacteria.

MALIGNANT CELL DEVELOPMENT.

The development of malignant (cancer) cells can also be viewed as a failure of the immune response.

In this case the failure to recognize and to respond properly to inappropriate cells within the body allows malignant cells to proliferate in an uncontrolled manner.

Kaposi sarcoma develops in many individuals with AIDS because the failing immune response | is unable to prevent malignancies.

ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS).

Acquired immunodeficiency syndrome or AIDS is caused by an infection with the human immunodeficiency virus (HIV).

This virus is able to adsorb specifically to lymphocytes through the CD_4 surface receptor.

Most of the lymphocytes with the CD_4 molecule are TH cells. HIV enters the TH cell via CD_{26}. Replication of HIV within TH cells leads to death of some of the infected cells, lowering numbers of T helper cells.

Nearly all the TH cells in blood, lymph nodes, and spleen are destroyed.

In addition, macrophages and microglial cells in the brain may become infected with HIV, causing dementia. Individuals with AIDS are subject to infection by a wide variety of disease-causing microorganisms and to the development of a form of cancer known as Kaposi sarcoma.

As the TH cells are killed, the ratio of Th to Ts cells decreases, from about 2.0 to less than 0.5.

This is because the TH cells decline from over 100,000/mL in healthy individuals to less than 50,000 /ml as a consequence of HIV infection. Because

individuals with AIDS have more T suppressor than Th cells, the immune response does not work efficiently.

This preponderance of T suppressor cells depresses other immune functions. When numbers of Th cells are decreased, B cells are not stimulated to produce sufficient numbers of antibodies to combat infections.

Amounts of lymphokines produced are insufficient to activate macrophages and cytotoxic T cells. Infected TH cells release soluble suppressor factor, which inhibits certain immune responses.

The T helper cells that survive do not have surface receptors for antigens. They are incapable of recognizing antigens. Thus the first step in the immune response is blocked.

Because HIV reduces the effectiveness of the immune system, the body is unable to rid itself of HIV once the infection is established.

The key to controlling this disease rests with prevention. HIV is transmitted by direct sexual contact, by exchange of blood, and from mother to fetus.

Casual contact does not result in transmission of the virus.

Sexually promiscuous individuals and intravenous drug abusers who share contaminated needles are at high risk of contracting this disease.

Steps have been taken to protect the blood supply used for transfusions. Blood is routinely tested for the presence of HIV.

Tissues for transplantation are also tested. Health care workers must take special precautions to avoid infection due to exposure to HIV-containing blood. Health care workers with AIDS have a special obligation to ensure that they do not transmit HIV to their patients.

Some drugs have proven effective in prolonging the life expectancies of AIDS patients by retarding the replication of HIV.

These drugs interfere with the replication of HIV. HIV is a retrovirus. Retroviruses carry out reverse transcription during replication; they copy their

RNA into DNA using a viral enzyme called reverse transcriptase. Azidothymidine (AZT) has been approved for treatment of AIDS.

At least 40 % of individuals treated with AZT develop intolerance and must cease taking the drug. Another drug dideoxyinosine (ddl), may be used as an alternate to AZT.

Both AZT and ddl block the formation of functional DNA during reverse transcription. AZT and ddl do not eliminate HIV.

They only slow down the rate of HIV replication and resultant destruction of T cells. |

There currently is no cure for AIDS. There is no vaccine for its prevention. Reducing the likelihood of exposure, such as by using condoms, is necessary to limit the spread of this disease.

As the disease progresses the immune system becomes less and less capable of defending the body against infection. Eventually the disease is fatal.

GENE THERAPY
WITH TUMOR INFILTRATING LYMPHOCYTES.

The cellular immune defense system recognizes abnormal cells when they arise in the body. It attempts to eliminate such cells. In this manner the immune system is able to hold in check most malignant (cancer-forming) cells when they occur.

T cells detect abnormal antigens on the surfaces of malignant cells and attack those cells. Sometimes the cellular immune response is adequate and malignant tumours do not develop.

In other cases the proliferation of malignant cells leads to the growth of cancerous tumours.

Recognizing that T cells have the capacity to attack malignant cells in the body, Stephen Rosenberg and colleagues at the National Institutes of Health

postulated that they could develop a method for cancer treatment based on the body's own immune response.

They sought to isolate T cells that could recognize specific types of malignancies. They then developed methods for culturing this specialized class of T ells, which they called tumour infiltrating lymphocytes (TIL-cells).

They hypothesized that if they could culture large numbers of TIL cells from a patient and could reinject the cultured TIL cells into that same patient, those TIL cells would then attack the developing malignant tumours.

The patient would be receiving ha or her own genetically modified cells.

In a number of cases where they earned out this procedure, there was remarkable regression of the tumours.

Some patients responded dramatically and the cancer went into total remission In other cases, however, the procedure failed to check tile growth of the tumours and the patients died of cancer.

It was not clear why the treatment worked in some cases and failed in others. Did the injected TIL cells survive in the body?

Did they reach the sites of tumours? Would the injection of lymphokins, such as interleukin, enhance the abilities of TIL cells to destroy malignant tumours? To answer these questions Rosenberg needed a method for tracking fee fate of the TIL cells that he had cultured and introduced back into the patient's body Rosenberg, with Michael Blaise and French Anderson, proposed to genetically label the TIL cells.

They obtained the gene for tagging the TIL cells from a bacterium. It is a gene that codes far neomycin resistance.

This gene does not occur naturally humans. Anywhere the neomycin resistance gene would be found in the patient could be directly tied to the introduced TIL cell.

The proposal to introduce genetically altered TIL cells into human subjects was reviewed by the Recombinant Advisory Committee of the National Institutes of Health.

The persons serving on that committee recognized the profound significance of the proposed experiments.

Not only could these experiments lead to improved cancer treatment, they also would pioneer the field of gene therapy.

It was clear that the next step in development would be to use genetic engineering to alter human cells to perform different functions.

Cells could be modified genetically and introduced into tile body to cure disease. After many long debates about the safety and scientific validity of the experiments, the Recombinant Advisory Committee approved tile experimental plan of Rosenberg, Blaise and Anderson.

Shortly thereafter, TIL cells obtained from several patients were marked with tile neomycin resistance gene and introduced back into those patients.

The researchers were able to follow the specific movement of the TIL cells that they had introduced.

They were able to improve the treatment regime *so* as to enhance survival of the introduced TTL cells.

Rosenberg, Blaise, and Anderson next proposed to genetically alter TIL cells by introducing the gene for tumour necrosis factor.

Lymphocytes that produce tumour necrosis factor are able to cause the shrinkage of malignant tumours. Again after extensive debates, the Recombinant Advisory Committee of the National Institutes of Health recommended that clinical trails of such recombinant cells be permitted.

These trials of represented the first true attempts at gene therapy. A new era m modem medicine based on recombinant DNA technology had begun.

A new treatment was added in the continuing battle against cancer.

AUTOIMMUNITY.

In some individuals the immune response fails to recognize self-antigens. In such cases the immune system attacks one's own body, a condition known as autoimmunity.

The inability to recognize self-antigens results in reactions that kill some of one's own cells.

There are a number of autoimmune diseases that result from the failure of the immune response to recognize self antigens. Such autoimmune diseases often result in the progressive degeneration of tissues.

Some autoimmune diseases affect single sites within the body. Graves disease, for example, is an autoimmune disease that affects the thyroid. In Graves disease the body produces an antibody that reacts with the receptor for thyroid stimulating hormone.

In contrast, some autoimmune diseases affect sites throughout the body. In systemic lupus erythematosus, numerous autoantibodies are produced that react with self-antigens.

They attack blood cells and cells at multiple body sites Antigen-antibody complexes circulate and settle in the glomeruli of the kidney.

In cases of myasthenia gravis, antibodies react with nerve-muscle junctions. In autoimmune haemolytic anaemia, antibodies react with red blood cells, causing anaemia.

Immunosuppressive substances are available to prevent the self-destruction of body tissues by the body's own immune response.

Various other disease conditions reflect the failure of the immune system to recognize self-antigens.

These self-antigens are similar to antigens associated with pathogenic microorganisms. For example, rheumatic fever is an autoimmune disease that results following an infection with group A streptococci *(Streptococcus pyogenes)* (FIG. 2).

Some antibodies made in response to group A streptococcal antigens can also react with myosin of the heart muscle tissue. After a strep throat, therefore, antibodies made against the group A streptococci cross react with myosin in some individuals, causing tissue damage to the heart.

These individuals develop rheumatic fever. Damaged heart valves may cause heart failure years later. The immune complexes between antibody and myosin or related antigens may also cause arthritis and kidney failure.

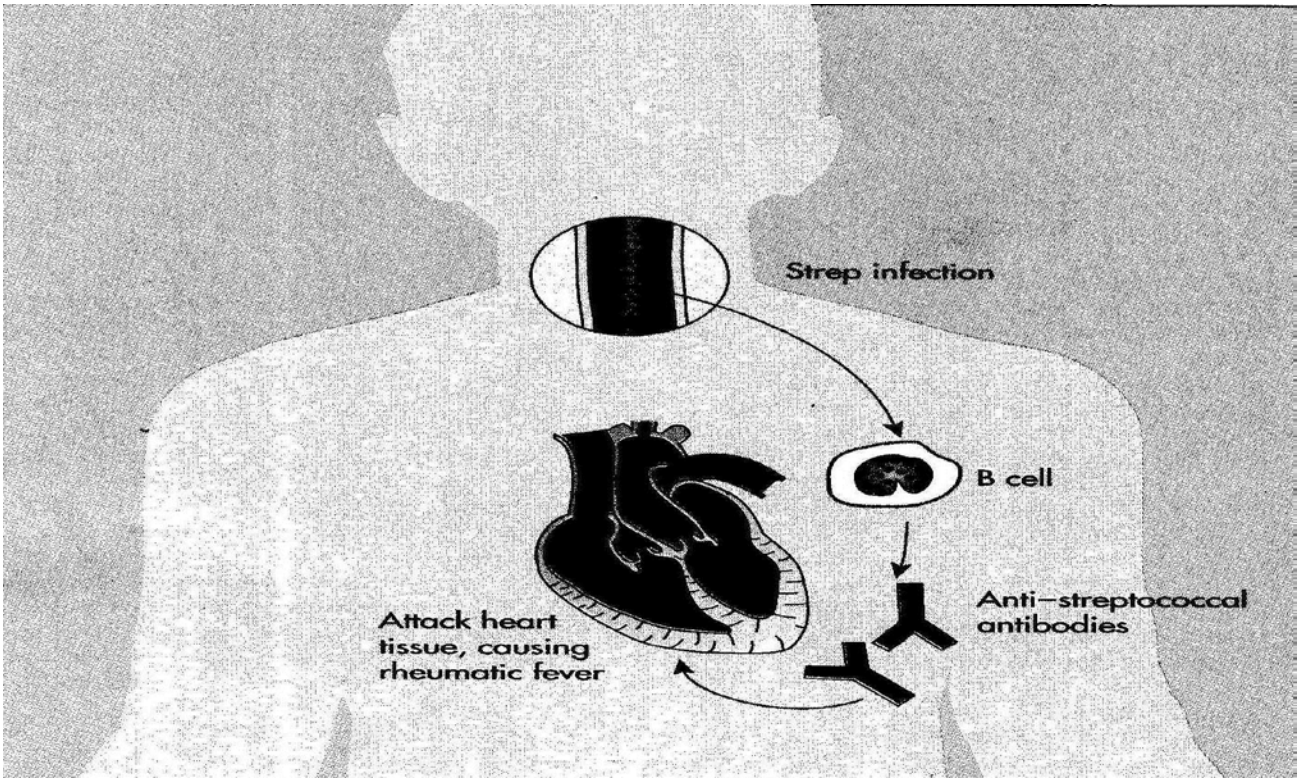


FIG. 2. An autoimmune response can occur following an infection with *Streptococcus pyogenes*.

The normal immune response produces antibodies against streptococcal antigens.

However, these anti-streptococcal antibodies can cross-react with heart tissue and cause damage that may result in later heart failure

Rheumatoid arthritis is a commonly occuring disease. Although rheumatoid arthritis is usually associated with older people, it often develops early in life.

It is a chronic inflammation of the joints, especially the hands and feet It can lead to crippling disabilities.

This form of arthritis often begins with a joint inflammation from an infection that causes phagocytic cells to release lysozymes.

These degradative enzymes attack and alter certain antigens. B cells make IgM antibodies in response to the antigens and cause more inflammation in the joints.

Treatment of rheumatoid arthritis is designed to relieve the symptoms. There is no cure. Hydrocortisone lessens inflammation and reduces joint damage.

Aspirin is also used because hydrocortisone produces side effects. Aspirin also reduces inflammation and pain.

Myasthenia gravis (MG) is an autoimmune disease that affects the neuromuscular system. It is characterized by weakness and rapid fatigue of the skeletal muscles.

It affects muscles in the limbs and the muscles used in eye movement, speech, and swallowing.

Patients with MG have a high incidence of thyroid abnormality, reduced levels of complement, and antiskeletal muscle antibody.

This disease is rare. It affects 3 persons in every 100/000. Twice as many women as men are affected.

The disease usually appears in late childhood to middle age.

Normal muscle contraction requires that pores in the membranes of neurons that stimulate muscles be open.

It appears that antibodies that react with self-antigens may be blocking these pores in people with miasthenia gravis. When the pores are blocked, the neurons do not release acetylcholine.

Acetylcholine initiates muscle cell contraction. Myasthenia gravis is treated with drugs that inhibit the enzyme that freaks down acetylcholine.

The slowing of acetylcholine breakdown allows each muscle longer time to act. This compensates for the decreased amount of acetylcholine.

Systemic lupus erythematosus is a widely disseminated, systemic autoimmune disease.

Erythematose means red *and* lupus means wolf. The name of the disease comes from a butterfly-shaped rash that appears on the nose and cheeks.

It was thought that the rash looked like *a* wolf bite. This disease occurs four times as often in women as in men, usually during the reproductive years.

Patients have reduced complement levels and high levels of immune complexes in their serum and glomeruli.

In this disease autoantibodies are made primarily against components of chromatin (DNA, RNA, and proteins).

Immune complexes are deposited between the dermis and epidermis and in blood vessels, joints, glomeruli of the kidneys, and central nervous system.

They cause inflammation and interfere with normal functions wherever they are. The symptoms depend on where the antigen-antibody complexes most interfere with function.

Usually there is inflammation of the blood vessels, heart valves, and joints. A skin rash appears.

Many victims die from kidney failure as glomeruli fail to remove wastes from the blood.

Patients with **Graves disease**, which include former President and Mrs. George Bush, suffer from overproduction of hormones produced by the thyroid. Normally the pituitary secretes thyroid-stimulating hormone, which controls the amount of thyroid hormone released.

Antibodies to thyroid-stimulating hormone receptors are produced in Graves disease patients.

These antibodies trigger thyroid cells to produce the hormones. The antibodies are not subject to hormonal feedback control and so the thyroid continues to produce, and overproduce, hormones.

Treatment of this disease involves destruction of part of the thyroid.

This is often accomplished using the radioisotope ^{n1}!, which is concentrated in the thyroid gland and subsequently kills thyroid cells.

Multiple sclerosis (MS) occurs in people 20 to 50 years of age. Common signs are sensory and visual motor dysfunction.

The etiology of this disease is unknown. It is generally believed, however, that MS is a T-cell-mediated autoimmune disease.

Macroscopic lesions called plaques are found in the central nervous systems of MS patients. The lesions contain macrophages and lymphocytes.

The term *multiple sclerosis* was originally used to describe the *wide* distribution of these lesions.

There is also breakdown in the myelin sheath that surrounds nervous tissue.

Summary

Immunodeficiencies

- An immunodeficiency is the result of an inadequate immune response.
- It can be inherited or acquired.

Severe Combined Immunodeficiency

- People with severe combined immunodeficiency disease (SCID) have neither functional B or T lymphocytes. They have no immunological response. Any infection can be fatal.
- Adenosine deaminase linked to polyethylene glycol (PEG-ADA) is used to treat SCID. Bone marrow transplants are also done.

DiGeorge Syndrome

• If the thymus does not develop properly, T cells are not differentiated and DiGeorge syndrome results

Bruton Congenital Agammaglobulinemia

• If B cells do not differentiate and produce antibodies, Bruton congenital agammaglobulinemia results. This condition affects only males.

• It is treated with IgG to maintain antibody levels in the circulatory system.

Late-onset Hypogammaglobulinemia

• Late-onset hypogammaglobulinemia is the most common immunodeficiency. Individuals with this condition are deficient in circulating B cells and/or B cells with IgG surface receptors.

Complement and Cellular Deficiencies

• If C3 complement is not produced, the body cannot defend against bacterial infections.

• Defective monocytes, neutrophils, and macrophages may cause immunodeficiencies.

Malignant Cell Development

• If the immune response system does not recognize and respond to the presence of abnormal cells, malignant cells can continue to grow.

Acquired Immunodeficiency Syndrome (AIDS)

• AIDS is caused by the human immunodeficiency virus (HIV). HIV destroys T helper cells. The proportion of T suppressor cells increases, which depresses immune functions.

B cells do not produce sufficient antibodies. Amounts of lymphokines are lowered.

• There is no cure for AIDS. Prevention is the only way to control it.

• Azidothymidine (AZT) and dideoxyinosine (ddl) slow the rate of HIV replication.

Autoimmunity

• Autoimmunity is the result of the inability of the body to properly recognize self-antigens. The immune system kills the body's own cells.

Rheumatoid Arthritis

• Rheumatoid arthritis is chronic joint inflammation. Lysozymes attack antigens and B cells make IgM in response, causing inflammation. There is no cure.

Myasthenia Gravis

• Myasthenia gravis affects the neuromuscular system. The pores in neuron membranes are blocked.

These neurons stimulate muscles. Blocked neurons do not release acetylcholine, which initiates muscle cell contraction.

Treatment is with drugs that inhibit the enzyme that breaks down acetylcholine.

Systemic Lupus Erythematosus

• Systemic lupus erythematosus is a systemic autoimmune disease. Autoantibodies are made against DNA components. The deposition of immune complexes causes inflammation.

Graves Disease

• Graves disease is an autoimmune disease of the thyroid in which antibodies to thyroid-stimulating hormone receptors are produced, allowing the overproduction of hormones.

Multiple Sclerosis

• Multiple sclerosis is believed to be a T-cell-mediated autoimmune disease. Lesions containing macrophages and lymphocytes are found in the central nervous system of people with MS.

II. Students' Practical Activities.

1. To read the results of the test of the determination of immune system state: a – radial immunodiffusion; b – E– and EAC–rosette formation; c – titer of the complement; d – titer of the lysozyme.

2. To solve the situation tasks.

1. To fill the card of primary examination of immune system and to choose the tests for its deep studying.

III. Tests and Assignments for Self–assessment.

2. It is necessary to estimate the immune status of the patient with chronic hepatitis.

A. What tests are necessary to use for determination of T lymphocytes activity?

3. The patient suffers for a long time for purulent lesion of the skin (piodermia). The doctor decided to estimate the state of B-cell immunity.

What tests can you propose for this purpose?

3. For individual with AIDS it is necessary to count up the interrelation between T-helpers and T-suppressers.

Explain, how it can be made?

4. The state of immunodifeciency was suspected in 2-years-old girl.

What signs of evaluation of B-cells and T–cells immunity will be used?

5. Explain what infectious diseases will appear in the organism with functional deficiency of:

a – antibody-mediated immunity; b – cell-mediated immunity.

5. What are the consequences of congenital deficiency of thymus?

6.

IV. The answers to the selfassessments.

1. E–rosette formation test, lymphocyte blast transformation test in the presence of phytohaemagglutinin.

2. The amount of different classes immunoglobulins, EAC – rosette formation test, lymphocyte blast transformation test in the presence of lipopolicaccharides.

3. Rosette formation test with erythrocytes which have IgM and IgG on their surface, the determination of lymphocytes with $CD3^{+}4^{+}8^{-}$ phenotype (Th cells) and $CD3^{+}4^{-}8^{+}$ phenotype.

4. E– and EAC–rosette formation test, quantity of immunoglobulins in blood serum, lymphocyte blast transformation test in the presence of phytohaemagglutinin and lipopolicaccharides.

5. A – diseases caused by different bacterial; B – viral diseases.

6. DiGeorge syndrome results from a failure of the thymus to develop correctly. Signs and symptoms of this disease often are apparent at birth.

They include deformities such as low-set ears, fish-shaped mouth, undersized jaw, and wide-set eyes. Elevated serum phosphate and low serum calcium also are characteristic of DiGeorge syndrome.

Individuals are prone to viral and other intracellular infections Avoidance of infecting agents is important in the management of patients with DiGeorge syndrome.

Children with DiGeorge syndrome that contract measles do not show the characteristic skin rash associated with this disease (the rash is due to activated T cells in the skin).

Because T helper cells are involved in enhancing antibody production by B cells, the antibody-mediated or humoral response is also depressed in individuals suffering from DiGeorge syndrome.

The complete absence of the thymus is rare Partial DiGeorge syndrome—in which some T cells are produced, although in lower numbers than in individuals with fully functional thymus glands — is more common.

Recommended reading list

Main literature

1. Ananthanarayan R. Textbook of Microbiology / R. Ananthanarayana, Jayaram CK. Paniker ; ed. by.: A. Kapil. - 9th ed. - India : Universities Press (Verlag), 2015. - 710 p.
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 : 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
5. Tropical Diseases : A Practical Guide for Medical Practitioners and Students / Y.A. Meunier, M. Hole, T. Shumba, B. J. Swanner. - OUP USA, 2013.

Informational resourses:

1. American Society for Microbiology — http:// asm.org.;

2. http://journals.asm.org; (American Society for Microbiology) — http:// asm.org.;
3. http://www.news-medical.net/health/Virus-Microbiology-(Russian).aspx;
4. http://www.rusmedserv.com/microbiology; http://www. rusmedserv.com/
5. http://rji.ru/immweb.htm; http://www.rji.ru/ruimmr;
6. http://www. infections.ru/rus/all/mvb journals.shtml;
7. http://dronel.genebee.msu.su/journals/ microb-r.html.
8. http://commons.wikimedia.org/wiki/Category:Medical_illustrations_by_Patrick _ Lynch.
9. http://www.nejm.org/doi/pdf/10.1056/nejmra064142
10. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3438653/
11. http://www.prb.org/pdf10/neglectedtropicaldiseases.pdf

http://www.who.int/neglected_diseases/diseases/NTD_Report_APPMG.pdf