МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ РЕСПУБЛИКИ БЕЛАРУСЬ

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Кафедра микробиологии, вирусологии и иммунологии

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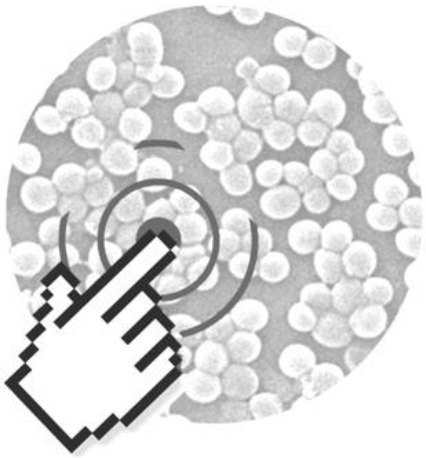
МЕДИЦИНСКАЯ МИКРОБИОЛОГИЯ И ИММУНОЛОГИЯ (тезисы лекций)

Учебно-методическое пособие
для студентов 2 курса факультета
по подготовке специалистов для зарубежных стран

MEDICAL MICROBIOLOGY AND IMMUNOLOGY (LECTURES ABSTRACTS)

The educational-methodical manual
for 2nd year students
of Faculty on preparation of experts for foreign countries



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Представленные тезисы лекций по микробиологии и иммунологии предназначены для студентов 2 курса факультета по подготовке специалистов для зарубежных стран. Рассмотрены вопросы морфологии, физиологии и генетики микроорганизмов, приведены сведения об общих механизмах функционирования системы иммунитета и современных иммунологических методах диагностики инфекционных и неинфекционных заболеваний.

Пособие может быть использовано для закрепления материала, изученного в курсе микробиологии, вирусологии, иммунологии.

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LIST OF ABBREVIATIONS

Ab – antibody
AMI – antibody-mediated immunity
ADCC – antibody-dependent cell-mediated cytotoxicity
APC – antigen presenting cell
BCR – B-cell receptor
C – complement
CD – cluster of differentiation
CMI – cell-mediated immunity
CFT – complement fixation test
CPM – cytoplasmic membrane
CTL – cytotoxic T-lymphocytes LPS – lipopolysaccharide
CIC – circulating immune complexes
Col-factor – colicins factor
CRP – C-reactive protein
DTH – delayed type of hypersensitivity
DDM – differential-diagnostic media
DNA – Deoxyribonucleic acid
dNTPs – deoxyribonucleotide triphosphates (A, C, G or T)
EB – elementary body
ELISA – Enzyme Linked Immune Sorbent Assay
F-factor – fertility factor
Fab – fragment antigen binding
Fc – fragment crystallized
Fc-R – receptor for Fc
GR – raft rejection
GIT – gastrointestinal tract
H-chain – heavy chain
Hfr-factor – high frequency recombination factor
H-Ag – flagella antigen
HIV – human immunodeficiency virus
IC – immune complex
Ig – immunoglobulin
IFN – interferon
IFA – immune fluorescent assay
IL – interleukins
IS-element – insertion sequence element

ITD – immediate type of hypersensitivity
J-chain – joining chain
K-Ag – capsule antigens
K-cells – killer cells
L-chain – light chain
LPS – lipopolysaccharide
MAC – membrane attacking complex
MPB – meat-peptone broth
MHC – major histocompatibility complex (HLA – human leukocytes antigens)
MRSA – Methicillin Resistant *Staphylococcus aureus*
MPA – meat-peptone agar
NA – nucleic acid
NK-cells – natural killer cells
O-Ag – O-antigen
PCR – polymerase chain reaction
Q-fever – “query” fever
RB – reticulate body
RIA – radio immune assay
R-colony – rough colony
RT – respiratory tract
RA – reaction of agglutination
RP – reaction of precipitation
RN – reaction of neutralization
R-factor – resistance factor
sIgA – secretary IgA
S-colony – smooth colony
tra-operon – transfer operon
TNF – tumor necrosis factor
Th – T-helpers
TCR – T-cell receptor
T – temperature
TB – tuberculosis
UV – ultraviolet
VRE – Vancomycin Resistant Enterococci

PART 1. GENERAL MICROBIOLOGY

Lecture 1. INTRODUCTION INTO MICROBIOLOGY. SYSTEMATICS, NOMENCLATURE AND CLASSIFICATION OF BACTERIA. DETECTION OF THE BACTERIA

Microbiology is a science studying the microorganisms.

Microorganisms are organisms, invisible by the unaided eye (microscopic object = microbe).

MICROBIOLOGICAL METHODS OF THE RESEACH:

1. **Bacterioscopical** (microscopical) method.
2. **Bacteriological** method (cultivation).
3. **Biological** method.
4. **Immunological** method (includes express-diagnostics, serotyping, serodiagnostics or serological method, skin testing or allergological method and methods of estimation of the immune status).
5. **Molecular-genetic method.**

OBJECTS OF THE MICROBIOLOGY:

1. **Eukaryotes** (fungi, some algae and protozoa).
2. **Procaryotes** (blue-green algae and bacteria).
3. **Viruses, prions** and **viroides**.

OBJECTS OF THE MEDICAL MICROBIOLOGY:

1. **Bacteria** (bacterial infections).
2. **Viruses** (viral infections).
3. **Fungi** (mycosis).
4. **Protozoa** (invasions).

TAXONS OF BACTERIA USED IN MICROBIOLOGY:

1. Kingdom (Prokaryote).
2. Division (see Bergy classification).
3. Order (name is ended with –ales).
4. Family (name is ended with –ceae).
5. Genus.
6. Species (basic taxon in microbiology).
7. Intraspecific variants or types (biovar/biotype, serovar/serotype, phagovar/phagotype, chemovar).

Strain (isolate) is a microbial culture, isolated from the certain source (soil, water, and human) and characterized differing from others within the species. **Pure culture** is a culture of the same microbes grown on the solid nutrient medium.

Serotype is an intraspecific variant of the bacterial species characterized by the certain set of antigens.

Phagotype is an intraspecific variant of the bacterial species characterized by certain sensitivity to the bacteriophages.

NOMENCLATURE OF BACTERIA:

Binomial system is used.

The first word in the name is the genus, with the first letter always capitalized. The second word is the species name, generally beginning from the small letter. For example, *Escherichia coli – E. coli*, *Staphylococcus aureus – S. aureus*.

BERGY CLASSIFICATION OF BACTERIA:

- Division **Firmicutes** (Gram-positive bacteria with the thick cell wall).
- Division **Gracilicutes** (Gram-negative bacteria with the thin cell wall).
- Division **Tenericutes** (Bacteria without cell wall – Mycoplasma).
- Division **Mendosicutes** (Archebacteria).

METHODS OF MICROSCOPY:

1. **Electron microscopy.**
2. **Light microscopy:**

- Basic light microscopy.
- Immersion microscopy (most frequently use in bacteriology).
- Dark-field microscopy.
- Phase-contrast microscopy.
- Fluorescent (luminescent) microscopy. Principle of immersion microscopy: immersion oil is placed between the glass slide and objective lens ⇨ eliminates losses of light rays getting in the objective lens.

METHODS OF STAINING:

1. **Simple staining** (staining by methylene blue or by aqueous fuchsine);
2. **Differential (complex) staining:**

- Gram staining (revealing of the cell wall structure).
- Ziehl-Neelsen staining (revealing of the spores or Acid Fast Bacteria/AFB);
- Neisser staining (revealing of the volutin granules of *Corynebacterium diphtheriae*).
- Burry-Hines staining (revealing of the capsules).
- Zdrodovsky staining (revealing of Rickettsia).
- Romanovsky-Giemsa staining (revealing of Spirochetes and the blood parasites).

Lecture 2. MORPHOLOGY OF THE BACTERIA. STRUCTURE OF THE BACTERIAL CELL. TAXONOMY, MORPHOLOGY AND DETECTION OF THE ATYPICAL BACTERIA

MORPHOLOGICAL FEATURES OF THE BACTERIA:

1. **Shape** and **size** of the bacteria.
2. **Arrangement** of the bacterial cells in a smear.
3. Structural components (organoids):

- **Obligate (basic):** nucleoid, cell wall, cytoplasmic membrane, mesosomes, ribosomes.
- **Facultative:** plasmids, inclusions, capsules, spores, flagella, fimbriae (pili).
- **Protective:** capsule and endospores.
- **Additional:** inclusions, flagella, pili (or fimbriae).

4. **Tinctorial properties** (ability to stain).
5. **Mobility** of bacteria.

SIZE OF BACTERIA:

- **Cocci** – 1 micrometer (1 mkm).
- **Rods:** very small (coccobacteria), small and average (most of the rods) and large (branch-forming and spore-forming rods).
- **Spirochetes** – thin and long bacteria.
- **Mycoplasma** – bacteria which have no constant size.

SHAPE OF BACTERIA:

1. **Cocci** (spherical bacteria).
2. **Rods** or **bacilli**.
3. **Helical** or **spiral forms** of bacteria (Vibrio, Spirilla, Spirochetes).
4. **Bacteria without the certain shape** (Mycoplasma).
5. **Bacteria with the filamentous shape** (Actinomycetes).
6. **Bacteria resemble viruses** (Rickettsia and Chlamydia).

ARRANGEMENT OF COCCI IN A SMEAR:

1. **Micrococci** (single cocci without special arrangement**).**
2. **Diplococci** (groups of two cells)**:**

- **pneumococci** (lanceolate or elongated diplococci);
- **gonococci, meningococci** (bean-shaped diplococci);
- **enterococci** (oval diplococci).

3. **Tetrads** (packet arranged from the number of cells, multiple to 4).
4. **Sarcina** (cubical packets of 8, 16, 32 cells).
5. **Streptococci** (chain of cells).
6. **Staphylococci** (groups of cocci resemble bunches of grapes)**.**

ARRANGEMENT OF RODS IN A SMEAR:

1. Rods without any order – **monobacteria** (most of the rods).
2. Pairs forming rods – **diplobacteria** (Klebsiella, Corynebacteria).
3. Chains forming rods – **streptobacilli** or **streptobacteria.**
4. Rods are arranged at the angles to each other (Corynebacteria).

SPIRAL BACTERIA:

1. **Vibrio** (comma shaped curved rods).
2. **Spirilla** (rigid spiral forms with few coils).
3. **Spirochetes** (flexuous spiral forms with many coils).

CELL WALL OF BACTERIA

• General structure: the main component of the bacterial cell wall is a **peptidoglycan**, which consists of two major subunits. Glycan portion (backbone) is composed of two amino sugars, N-acetylmuramic acid and N-acetylglucosamine. The peptide portion consists of a chain of several aminoacids, which join the glycan backbones.

• **Gram staining** is a method of revealing of the cell wall structure. Steps: staining by gencian violet, treatment by iodine solution, alcohol decolorization, staining by fuchsine. *Result of Gram staining: Gram-positive bacteria are violet, Gram-negative bacteria are pink.*

Table 1 — Morphological groups of the bacteria according to the Gram state

Gram-positive group of bacteria	**Gram-negative group of bacteria**
1. **Majority of cocci:** Streptococci, Staphylococci, Pneumococci	**1. Exclusion from cocci:** Gonococci and Meningococci
2. Spore-forming rods: – Bacilli – Clostridia	**2. Non spore-forming rods**: – Enterobacteria (*E. coli*, Salmonella, Shigella, Citrobacter, Serratia, etc.) – Coccobacteria (Yersinia, Bordetella, etc.)
3. Rods with irregular form: Corynebacteria and Mycobacteria	**3. Spiral forms of bacteria:** Spirochetes, Spirilla, Vibrio
4. Rods with regular form: Listeria	**4. Bacteria which are resemble viruses:** Rickettsia and Chlamydia
5. Branch-forming rods: Actinomycetes	**5. Bacteria lost cell wall** (Mycoplasma) and **bacteria with defect of cell wall** (L-forms)

• **Cell wall of Gram-positive bacteria:**

1. Many layers of the peptidoglycan (thick cell wall).
2. Teichoic acids and lipoteichoic acids.

• **Cell wall of Gram-negative bacteria:**

1. Single layer of the peptidoglycan (thin cell wall).

2. Periplasmic space (periplasm).

3. Outer membrane (includes phospholipids, lipoproteins, and most important component LPS or lipopolysaccharide).

4. Porins (proteins).

• **LPS** consists of **O-antigen** (O-Ag), **core polysaccharide** and **lipid A** (toxic component of LPS → second name of LPS is endotoxin).

• **Cell wall-less forms** of bacteria:

1. **L-forms** are the bacteria which lack cell wall but can grow and divide (L-transformating agent are antibiotics, lysozyme, aminoacids, physical factors; L-forms may produce chronic or persistent infection);

2. **Protoplasts** are Gram-negative bacteria which lack all cell wall (can't dividing); **Spheroplasts** are Gram-positive bacteria which lack most of cell wall (also can't dividing).

FLAGELLA AND BACTERIAL MOTILITY

• **Flagella** are the filamentous structures attached to the cell surface that provide the swimming movement of bacteria.

• **Spirochetes** have periplasmic or internal flagella (axial filaments).

• **Composition:** protein **flagellin**.

• The presence or absence of flagella and their number are characteristics of different genus of bacteria.

• Cocci are immobile bacteria. Spiral bacteria are mobile. Rods can have different mobility (*E. coli* is mobile, Shigella are immobile).

Classification of bacteria (rods) according to number and localization of flagella:

1. **Monotrichous** (polar flagella, single).

2. Polytrichous (many flagella):

• **amphitrichous** (flagella at both poles of the bacterial cell);

• **lophotrichous** (tufts of flagella localized at the end of the cell);

• **peritrichous** (flagella are arranged all round the cell).

3. **Atrichous** (lack of a flagella):

• Flagellum consists of several rings embedded in the cell envelope (basal body), hook-like structure and flagellar filament. The inner rings (M- and S-rings), located in the plasma membrane. The outer rings (P- and L-rings), located in the periplasm and the outer membrane respectively.

• **Detection of the flagella and bacterial motility**:

1. Flagellar staining (impregnation method).

2. Electron microscopy.

2. Motility test medium demonstrates if cells can swim in a semisolid medium (spreading type of bacterial growth).

3. “Hanging drop” method or “pressed drop” method (demonstrates the motility of the microorganisms).

FIMBRIAE (PILI)

• **Fimbriae** are short, hair-like structures on the surfaces of bacteria. Like flagella, they are composed of protein (**pilin**).

• Fimbriae are very common in Gram-negative bacteria, but occur in some Gram-positive bacteria as well.

• **Function:** the major factor of bacterial virulence because they allow pathogens to attach and colonize the tissues and/or to resist attack by phagocytes.

• Types of fimbriae: **F-pili** or **sex pili** (mediate conjugation) and **common pili** (almost always called **fimbriae**) are usually involved in the specific adherence.

CAPSULE

• **Capsule** is a layer outside of the cell wall.

• Composition: polysaccharide or polypeptide.

• Type of capsule: **microcapsule** (produced by the most of bacteria) and **macrocapsule** (some bacteria).

• Capsules are most pronounced in such **bacteria**:

- klebsiella (always form the capsules even when growing on media);
- pneumococci, meningococci;
- bacilli causing anthrax;
- many coccobacteria.

• Functions: **adherence** of the bacteria and **protection** of the bacteria from the immune factors (phagocytosis and antibodies).

• Method of revealing is **Burry-Hines staining**. Steps: Indian Inc for background, then - fuchsine. *Result of Burry-Hines staining: dark background, capsules are colorless, bacteria inside of capsules are pink.* After other **simple** or **complex staining** the capsules are visible as <u>colorless areas around bacteria</u>.

SPORES OF BACTERIA

• **Endospore** is a resting form of the bacteria for defence in the unfavourable conditions of external environment.

• **Exospores** are the reproductive structures in streptomycetes.

<u>Differences of the exospores in compare with endospores</u>:

1. Not resistant in unfavorable conditions of environment.
2. Forms outside of the bacterial cell.
3. One bacterial cell contains many exospores.

• **Composition** of endospores: DNA, cytoplasm with some enzymes, plasma membrane, spore wall (normal peptidoglycan), cortex (tick layer of peptidoglycan), and keratin spore coat (exosporium).

• **Resistance** of endospores is provided by:

1. Practical absence of unbound water.
2. Increased calcium concentration.
3. Presence of dipicolinic acid.
4. Especial composition of a protein.
5. Especial composition of the cortex:

• Conditions for sporulation: external environment and artificial media.

• Spore-forming bacteria:

– **bacilli** (with central, small oval endospores);

– **clostridia** (with central/subterminal/terminal, big, spherical endospores).

• Method of revealing is **Zhiel-Neelsen staining**. Steps: Zhiel fuchsine and heating of the smear, decolorization by acid, staining by methylene blue. *Result: spores are pink, vegetative cells are blue.*

ATYPICAL BACTERIA

SPIROCHETES

1. Taxonomy: three genera – **Treponema** (→ endemic treponematoses and epidemic syphilis), **Borrelia** (→ borrelioses: epidemic and endemic relapsing fever, Lyme borreliosis**),** and **Leptospira (**→ leptospirosis**)**.

2. Morphology: Gram-negative spiral forms of bacteria, motile. Treponema has 8-12 regular coils, Borrelia – 3-8 irregular coils, Leptospira – many primary coils, few secondary coils, shape like C or S and hooks like ends. They are facultative intracellular parasites.

3. Ultrustructure: outer membrane contains many lipids, there are several **axial filaments** (internal flagella or periplasmic flagella) in a periplasm, and their function is mobility.

4. Methods of revealing:

• study of mobility with using dark field microscopy (“hanging drop” method);

• **romanovsky-Giemsa staining** (Treponema – pink; Leptospira – red; Borrelia – blue).

ACTINOMYCETES

1. Taxonomy: three genera – **Actimyces** (→ actinomycosis), **Streptomyces** (→ mycetoma, or fungus tumor) and **Nocardia** (→ nocardiosis).

2. Morphology: Gram-positive brunch-forming rods, nonmotile, can produce exospores. Actinomyces can form microcolonies – yellow sulfur granules (**druses**) in the pus during the disease (center of a druse is gram-positive, peripheral part is gram-negative).

3. Methods of revealing:

• Gram staining.

MYCOPLASMA

1. Taxonomy: two genera – **Mycoplasma** (→ mycoplasmosis and pneumonia, and **Ureaplasma** (→ ureaplasmosis).

2. Morphology: smallest free-living bacteria, **“membrane parasites”**. Gram-negative, cell wall-less polymorphic bacteria, nonmotile. They have three-layered plasma membrane which includes sterols (unlike other bacteria).

3. Methods of revealing:

- Phase-contrast microscopy.
- Electron microscopy.

RICKETTSIA

1. Taxonomy: genera **Rickettsia** (→ rickettsioses: epidemic and endemic typhus, spotted fever, scrub typhus, etc.); **Bartonella** (→ trench fever); **Coxiella** (→ Q-fever).

2. Morphology: Gram-negative smallest polymorphic bacteria (rods or coccobacteria). They are **obligate intracellular parasites** (resemble viruses), can’t produce NAD and glycolytic enzymes.

3. The Rickettsia frequently has a close relationship with **arthropod vectors** (ticks, lice) that may transmit the organism to mammalian hosts. Rickettsia must be grown in the laboratory by *cultivation in chicken embryo, cell culture or laboratory animals* and they have not been grown in artificial medium.

4. Methods of revealing:

- romanovsky-Giemsa staining (dark blue rods on the light blue background of the host cell);
- zdradovsky staining (pink rods on the blue background of the host cell).

CHLAMYDIA

1. Taxonomy: genera **Chlamydia** (→ trachoma, chlamydiosis, “inclusion” conjunctivitis) and **Chlamydophila** (→ pneumonia, bronchitis).

2. Morphology: Gram-negative smallest polymorphic bacteria (cocci). They are **obligate intracellular parasites** (resemble viruses), they can’t produce ATP (chlamydia are “energy parasites”).

3. Chlamydia can produce **intracellular inclusion bodies** (microcolonies) near the nucleus inside of the host cells.

4. Chlamydia is unique bacteria – they have **cycle of development** (life or growth cycle). There are revealed infectious elementary body (EB) which develops into a noninfectious reticulate body (RB) within a cytoplasmic vacuole in the infected cell. The RB divides by binary fission to form particles which, after synthesis of the outer cell wall, develop into new infectious EB progeny. The yield of chlamydial EB is maximal 36 to 50 hours after infection.

4. Methods of cultivation and revealing of Chlamydia are the same as for Rickettsia. Only **cytological method** can be used for revealing of the inclusions. Also can be used direct **IFA** for detection the inclusion antigens.

Lecture 3. PHYSIOLOGY OF THE PROCARYOTES. PLASTIC AND ENERGY METABOLISM. CULTIVATION OF BACTERIA

PHYSIOLOGY OF MICROORGANISMS includes:

- types of microbes nutrition;
- types of microbes respiration;
- cultivation of bacteria;
- biochemical activity of bacteria;
- variability of bacteria;
- formation of toxins and other factors of pathogenicity;
- sensitivity to antibiotics, bacteriophages, bacteriocins;
- others biological properties.

Bacterial metabolism is a combination of the physical and chemical processes providing living activity of a microbial cell. Metabolism consists of two main directions: **anabolism** (biosynthesis of polymeric compounds – proteins, NA, polysaccharides – from monomers) and **catabolism** (biodisintegration of complex polymeric compounds).

NUTRITIONAL REQUIREMENTS

1. **Carbon** (is required for all bacteria growth):

a) autotrophs (use carbon dioxide as source of carbon);

b) heterotrophs (use organic compounds as source of carbon).

2. **Inorganic ions**:

a) halotolerant bacteria (resistant to salts);

b) halophilic bacteria (require for growth high concentration of salts).

3. **Growth factors** (vitamins, purines, pyrimidines, and aminoacids):

a) prototrophs (wild strains which don't require growth factors);

b) auxotrophs (mutant strains which need growth factors).

4. **Electron donors**:

a) litotrophs **(**use reduced inorganic compounds as source of energy);

b) organotrophs (use reduced organic compounds).

5. **Electron acceptors** play essential role in respiration and fermentation:

a) oxygen is terminal electron acceptor in aerobic respiration;

b) pyruvate, lactate and other organic compounds are products of the terminal electron acceptance in fermentation.

OXYGEN REQUIREMENTS

1. **Obligate aerobes** (require oxygen).

2. **Obligate anaerobes** (oxygen inhibits their growth).

3. **Facultative anaerobes** (able use both electron acceptors – oxygen and organic compound, can have aerobic respiration and fermentation).

4. **Microaerophilic bacteria** (grow best under decreased oxygen tension).

5. **Capnophilic bacteria** (grow best under increased carbon dioxide tension).

6. **Aerotolerant bacteria** (can survive, but do not grow for a shot period of time in the presence of oxygen).

Tolerance to oxygen is related to presence of the enzymes:

– **Catalase** (breakdown hydrogen peroxide).

– **Superoxide dismutase** (neutralize the toxic oxygen radicals).

ENERGY METABOLISM

1. **Fermentative metabolism** (use the organic compounds as both electron donors and electron acceptors, includes Glycolytic pathway, Entner-Duodoroff pathway and Pentose phosphate shunt).

2. **Respiratory metabolism** (use aerobic respiration).

3. **Autotrophic metabolism** (use photosynthesis and anaerobic respiration).

PLASTIC METABOLISM

1. Aminoacids biosynthesis.
2. Nucleotide biosynthesis.
3. Macromolecules biosynthesis (RNA, DNA, proteins).
4. Peptidoglycan biosynthesis.

GROWTH

Factors affecting growth of bacteria in the laboratory:

1. Media (nutritive media).

Classification of media:

a) *On consistency*:

- liquid media (MPB – meat-peptone broth);
- semisolid media (semisolid MPA);
- solid media (MPA – meat-peptone agar).

b) *On composition*:

- simple media (MPB and MPA);
- complex media;
- synthetic (or semisynthetic) media;
- natural media.

c) *On destination*:

- **fundamental (basic) media** – MPA, MPB, *blood agar* (for cultivation of the unpretentious bacteria);
- **selective media** (for pure culture getting only of the certain bacterial species; for example, use of salt agar for Staphylococci);
- **differential-diagnostic media** – DDM or indicator media (for getting pure cultures and simultaneous differentiation of the different species by their biochemical activity; for example Endo agar is used for all Enterobacteria, but *Escherichia coli* gives lactose-fermenting pink colonies while Shigella dysenteriae gives lactose-nonfermenting colorless colonies).
- **Enrichment media** (for accumulation of the certain groups of the bacteria in the pathological material before inoculation).
- **Transport media.**

2. Temperature:

a) methophilic bacteria (grow best at 20–40°C, optimal t=37°C);

b) thermophilic bacteria (grow best at 50–60°C);

c) psychroplilic bacteria (grow best at 10–30°C).

During typical bacterial growth bacterial cells divide by a binary fission and their mass and number increase in the exponential manner.

Bacterial growth curves consist of several stages:

1. **Lag phase** (intense physiologic adjustment).
2. **Log phase** (maximal rate of cell division).
3. **Stationary phase** (balance between cell growth and cell death, sporulation).
4. **Decline or death phase** (bacterial cells undergo lysis).

Microbiological (bacteriological) method of investigation (stages):

1. **First day of investigation:**

- preliminary microscopy of the pathological material;
- inoculation of the pathological material on an agar plate ► to get **isolated colonies** (obtaining of the pure culture).

2. **Second day of investigation:**

- study of the cultural characteristics of the colonies: shape, size, surface (R- and S-colonies), consistency, pigmentation, opacity, elevation, edges, etc.
- re-inoculation of the suspected colonies on a slant agar ► for accumulation of pure culture.

3. **Third day of investigation:**

IDENTIFICATION of pure culture includes:

- Check of purity of a pure culture (study the **morphological properties**).
- Study of the **biochemical properties** of bacteria (revealing of the proteolytic and saccharolytic enzymes, catalase test, oxidase test, etc.).
- Estimation of a bacterial **antigenic structure** (detection of serotypes).
- Study of the bacterial **sensitivity to bacteriophages** – viruses of bacteria (detection of the phagotypes).
- Revealing of the **pathogenic factors** of bacteria.
- Study of the bacterial **sensitivity to antibiotics**…

CULTIVATION OF THE ANAEROBES

There are several **techniques for obtaining anaerobic conditions**:

- GasPak anaerobic jar (anaerostat) and “Genbags”.
- Gas generating boxes (“Genbox anaer”).
- Reducing agents in culture media.
- Boiling of nutrient broth and layer over it by sterile vaseline.
- Combined cultivation of aerobes with anaerobes on the same Petri dish.

Media for anaerobes:

- **Kitt-Tarozzi medium** (cooked meat glucose medium).
- **Blood-sugar agar** (Zeisler agar).
- **Bismuth-sulphite agar** (Wilson-Bler medium).
- **Thioglycolic medium** (medium for control of sterility).

Lecture 4. BACTERIOPHAGES. GENETICS OF THE BACTERIA

BACTERIOHGES

Bacteriophages (phages) are the bacterial viruses. Nomenclature of phages is based on the name of the host which is sensitive to definite phage. Structure of phages – **nucleic acid (DNA or RNA)** and **proteins,** type of symmetry of the phages is **complex** (head has cubical, tail has helical symmetry)**,** but it is **simple viruses** (have no lipid envelope).

The main properties of bacteriophages:

1. Obligate specificity.
2. They more tolerant to the high temperatures than viruses.
3. Stability to the antibiotics, disinfectants and antiseptics.
4. Stability to the high pressure.
5. The proteins of the phages cause the production of antibodies.

Structural components of bacteriophages:

- Head (with NA).
- Collar.
- Tail and tail fibers.
- End plate (base plate).

Morphological types of phages:

1. Only tail.
2. Only head.
3. With short tail and head.
4. With long unretractile tail and head.
5. With long retractile tail and head.

Types of life cycle of phage:

- **Virulent or lytic cycle** (intracellular multiplication of the phage is finished by the lysis of the host bacterium and the release of progeny virions ►name of such phages is **virulent phages**).

- **Temperate or lysogenic cycle (**phage DNA becomes integrated with the bacterial genome ► name of such phages is **temperate phages).**

Lytic cycle of bacteriophages:

1. The first step in the replication of the phage in its host cell is called adsorption. The phage adheres to bacteria by means of its tail fibers.

2. Phage injects its DNA into the bacterial cell. This process is called penetration.

3. Synthesis of phage proteins and genomes.

4. Assembly of the phage progeny.

5. Lysis of the host cell and the release of the progeny.

Lysogenic cycle of bacteriophages:

After penetration, the phage DNA integrates into the bacterial chromosome. Temperate viruses usually do not kill the host bacterial cells they infect. These bacteria are called **lysogenic**. The virus in this state is called **prophage**.

Usually it is difficult to recognize lysogenic bacteria because lysogenic and nonlysogenic cells appear identical. But in a few situations the prophage supplies genetic information such that the lysogenic bacteria exhibit a new characteristic (new **phenotype**), not displayed by the nonlysogenic cell, a phenomenon is called **lysogenic conversion**. Hence, *Corynebacterium diphtheriae* can only produce the toxin responsible for the disease if it carries a temperate virus called phage beta.

Classification of the bacteriophages according to the spectrum of their action:

- Polyphages – infect several species of bacteria.
- Monophages – infect one species of bacteria.
- Type phages – infect only a certain phagotype of bacteria.

Practical application of the phages in medicine:

1. **Phage typing** (phagotyping) is detection of bacterial phagotype of the pure culture with help of the typical bacteriophages.
2. **Phagotherapy** and **phagoprophylaxis** of some bacterial infections.

ORGANIZATION OF THE GENETIC MATERIAL OF THE BACTERIA

- **Nucleoid** (bacterial chromosome) is a molecule of DNA in cytoplasm which codes vital information for bacteria.
- **Extra-chromosomal factors** code the information which is not important for life.

Extra-chromosomal factors include:

1. Autonomous factors (**plasmids**) ► its replication is independent.
2. Non-autonomous factors (*integrated* into bacterial nucleoid or plasmids):

– **transposones;**

– **IS-element** (“insertion sequences”).

Transposones and IS-elements are nucleotides which can change their localization in the bacterial genetic material – nucleoid or plasmids **(**“jumping genes” of genome), function – change the bacterial genome resulting in the induction of the mutations.

PLASMIDS are small molecules of DNA in a bacterial cytoplasm.

Functions of the plasmids:

1. **Regulatory** (help for nucleoid).
2. **Coding** (introduce new information into the bacteria).

Location of plasmids: autonomous and integrated in to the nucleoid.

Classification of plasmids:

- **F-plasmid** (provide conjugation in bacteria).
- **R-plasmid** (provide multiple resistance to the antibiotics).

• **Hly-plasmid** (provide synthesis of hemolysins).

• **Ent-plasmid** (provide synthesis of enterotoxins).

• **Plasmids of bacteriocinogenicity** (provide synthesis of the bacteriocins).

F-PLASMID (F-factor)

Sex (**f**ertility) factor provides **conjugation** in bacteria by forming of **conjugative pili** between the mating bacteria.

F-plasmid contains only **tra-operon** which is need for plasmid transmission (other genes are not presented generally).

Location F-plasmid in bacterial cell:

1. **Integrated** into bacterial genome (**Hfr-plasmid** – provide high frequency of recombination between bacteria during conjugation).

2. **Autonomous** F-plasmid.

Cell which have F-plasmid are called **male cells** (**F^+-cells or Hfr-cells**). These cells have conjugative pili on their surface! Male cells are the **donor cells** in conjugation. Cells without F-plasmid are the female cells (**F^--cells**), have no conjugative pili. Female cells are **recipient cells** in conjugation.

R-PLASMIDS

• Plasmids coding multiple resistance to antibiotics.

• Ways of transmission: by **transduction** (in Gram-positive bacteria) or by **conjugation** (in Gram-negative bacteria).

• Composition:

– r-operon + tra-operon (conjugative plasmids);

– only r-operon (nonconjugative plasmids).

PLASMIDS OF BACTERIOCINOGENECITY

Bacteriocins are antibiotics' like substances which one bacterial species can produce against closely related species. Ex., *E.coli* can produce colicins against the enteric pathogens (name of plasmid – **col-factor**).

Properties of bacteriocins:

1. Bacteriocins resemble the antibiotics with narrow spectrum of action.
2. Bacteriocins are causing the destruction of the target cells.
3. After the releasing of bacteriocins the cell will die.

Properties of col-factor:

a. Rarely integrate into nucleoid.
b. Usually exist in the repressed state.
c. Potentially lethal plasmid.

Composition of col-factor:

• tra-operon;

• genes coding synthesis of colicins.

Importance of colicins for medicine: normalization of normal flora of an intestine (preparation is called **colibacterin**).

VARIABILITY OF BACTERIA

Types:

1. **Phenotypic** (nonheritable, modification) variability;
2. **Genotypic** (heritable, genetic) variability:

– mutation variability;

– recombinant variability.

3. **SR-dissociation.**

Phenotypic variability is changes affecting only the **phenotype** of bacteria. Modifications of bacteria have certain properties:

► not stable and usually could be lost very quickly;

► may be morphological, biochemical, antigenic, etc.

Mutation variability is changes which occur in the primary structure of DNA molecule. Mutagens are chemical substances or physical factors which cause mutations in DNA structure of bacteria.

Classification of mutations:

– Insertions
– Deletions
– Direct
– Reverse
– Lethal
– Spontaneous
– Inductive
– Morphological
– Biochemical
– Physiological

Recombinant variability is changes in DNA structure occurring as a result of integration of the part of DNA of recipient cell into DNA of donor cell.

Result of the recombinations is a formation of the **recombinant cells** which are cells with new genes and new properties!

Forms of the recombinant variability (recombinations):

1. Transformation.
2. Transduction.
3. Conjugation.

Transformation is a direct transfer of genetic material from the donor cell to the recipient cell (or uptake of the naked donor DNA fragments by the recipient competent cell).

Factors affecting transformation:

1. **DNA size state**: Double stranded DNA of works best.
2. **Competence** of the recipient: the bacteria are said to be *competent* when they are able to take up DNA from outside.

Steps in transformation:

1) uptake of DNA;
2) homologous recombination between recipient genes and donor genes.

Transduction is a transfer of genetic material from donor cell to recipient cell with help of the temperate defective bacteriophages. Often result of transduction is a lysogenic conversion.

Types of transduction:

1. Generalized transduction is a transduction in which potentially *any* bacterial gene from the donor can be transferred to the recipient.

2. Specialized transduction is a transduction in which only *certain* donor genes can be transferred to the recipient.

3. **Abortive transduction** occurs when the new DNA does not integrate into the chromosome and is eventually lost.

Conjugation is a transfer of genetic material from donor cell to the recipient cell through conjugative pili.

Mating types in bacteria:

1. F^+-cells.
2. Hfr-cells.
3. F^- - cells.

SR-dissociation is an appearance of R-form and S-form of bacteria.

Table 2 – Comparison of R-form and S-form of bacteria

S-form	R-form
Smooth colony	Rough colony
Usual virulent	Usual nonvirulent (exception *B. anthracis, Y.pestis, M.tuberculosis*)
Capsule and flagella are present	Capsule and flagella are not present
Sensitive to bacteriophages	Less sensitive to bacteriophages
Biochemically active	Biochemically less active
Complete set of antigens	Incomplete set of antigens
More resistant inside of the host to phagocytosis and antibodies	More resistant to unfavorable conditions of environment

Molecular-genetic methods applied in microbiological diagnosis:

- Genetic engineering.
- DNA-probe.
- Molecular hybridization.
- Blotting techniques.
- Polymerase chain reaction (PCR).

Polymerase chain reaction

PCR is used to amplify a specific region of a DNA strand (the DNA target). **Amplification** is multiple coping of DNA in vitro. Basic PCR set up requires several components and reagents. These components include:

- **DNA template** that contains DNA region (target) to be amplified;
- **two primers** that are complementary to the ends of DNA target;
- **taq polymerase** (enzyme for replication);
- **nucleotides** (dNTPs).

PCR is commonly carried out in small reaction tubes in a thermal cycler. The thermal cycler heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction (see below).

Typically, PCR consists of a series of 20–40 cycles. Each cycle consists of several steps: 1) *Denaturation step*: This step consists of heating the reaction to 94–98°C for 20–30 seconds. It causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules; 2) *Annealing step*: The reaction temperature is lowered to 50–65°C for 20–40 seconds allowing annealing of the primers to single-stranded DNA template. Polymerase binds to the primer-template hybrid and begins DNA replication; 3) *Extension/elongation step*: Optimum activity temperature is 72°C used for Taq polymerase. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template. The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified.

PCR in diagnosis of diseases

PCR permits early diagnosis of malignant diseases PCR also permits identification of non-cultivatable or slow-growing microorganisms such as mycobacteria, anaerobic bacteria, or viruses. The basis for PCR diagnostic applications in microbiology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes. The high sensitivity of PCR permits virus detection soon after infection and even before the onset of disease. Such early detection may give physicians a significant lead in treatment. The amount of virus ("viral load") in a patient can also be quantified by PCR-based DNA quantitation techniques.

Lecture 5. ECOLOGY OF THE BACTERIA. HUMAN NORMAL FLORA

Ecology is the study of the relationship of organisms to each other and to their environment. Different bacteria are capable of growing in a wide variety of environments but each will grow best in those environments for which it is well adapted.

Ecosystem is biological community including humans and microorganisms.

Population is totality of same species microbes living on the certain territory (biotope).

Biotope is area of the biosphere with same conditions for life.

Biosphere is region of earth inhabited by living organisms.

Bacteria can have a full range of **symbiotic interactions** with their animal hosts.

Symbiosis is living together of two dissimilar organisms (symbions).

Ecological connections between symbions**:**

1. **Mutualism** is an association in which both partners benefit.

2. **Parasitism** is a relationship between two organisms in which one organism (parasite) uses other organism as a host for nutrition and replication. **Parasites** are microbes that can multiply inside the host.

Commensalism is a relationship between parasite and host in which they live in a complete harmony without causing any damage for host (ex.: normal flora). Many commensals behave as **facultative pathogens** (or **opportunistic pathogens**) in that they can produce disease when the host resistance is lowered.

Obligate (true) parasitism is a relationship in which parasite cause damage to the host. Obligate parasites are mainly **obligate pathogens**.

Steps of the development of parasitism:

1. **Saprophytes:**

They are free-living microbes that subsist on dead or decaying organic matter in nature (soil, water). They are generally incapable to multiply in the living tissues. Exceptionally, some saprophytes (like *Bacillus subtilis*) may infect only the immunocompromised persons.

2. **Facultative parasites** (can live a long time as saprophytes);

3. **Obligate parasites** (live mainly in a host):

- facultative intracellular **parasites**;
- obligate intracellular **parasites.**

Sanitary microbiology

Soil contains a large variety of microorganisms that are able to degrade or chemically modify organic and inorganic molecules. Soil environment includes different forms of life like **bacteria** (most important – **actinomycetes** and **myxobacteria**), algae, **fungi** (**Candida, Aspergillus, Penicillium,**

Rhizopus, and **Mucor** are most common), protozoa, nematodes, worms and other eukaryotic organisms.

Air contains **Sarcina, Bacilli**, **actinomycetes, fungi.**

Aquatic environment contains aerobic cocci, Leptospira, Pseudomonades, Proteus, anaerobic bacteria.

NORMAL FLORA

Normal flora (normal microbial flora, microflora, microbiota) is a group of the microorganisms that colonize the body surfaces but does not usually cause a disease.

- May be residents (constant) and transients (occasional).
- Constant for each species.
- 400 species of normal flora!

Ecological position of normal flora: commensals and parasites.

Localization of a normal flora – skin, mucous, conjunctiva, upper respiratory tract, lower urogenital tract, almost all gastrointestinal tract.

Composition of normal flora:

Skin – Staphylococci, Diphtheroids, Candida, Propionebacteria.

Upper respiratory tract – greenish Streptococci, Staphylococci, Diphtheroids, Pseudomonades (larynx, trachea, bronchi, lung do not contain normal flora!).

Oral cavity normal flora – Staphylococci, Streptococci, Candida, Lactobacteria, Spirochetes, Neisseria, Peptostreptococci, Bacteroides.

Colon normal flora – Bacteroides and Bifidobacteria (96-99 %), E. coli, Enterococci, very small amount of Staphylococci, Clostridia, Candida, Proteus, and also Enterobacter, Citrobacter, Klebsiella.

Female genital tract – Lactobacilli, some Streptococci, Candida.

Male urethra is relatively sterile.

Role of normal flora:

- Prevent colonization of body by other pathogens.
- Stimulation of immune system (normal flora have related antigens with pathogens).
- Intestinal flora produce vitamins (B, K).
- Some species produce antibiotic substances (colicins).
- Digestion of food in the colon.
- Neutralization of endogenous or exogenous toxic substances.

Normal flora as pathogens

Most species of normal flora are the **facultative pathogens**, some species are **nonpathogenic**, and some ones are the **obligate pathogens**.

Normal flora can produce infections (can be named so: opportunistic infections, endogenous infections, autoinfections, secondary infections, etc.) at

certain conditions: immunocompromised status, antibacterial therapy, penetrating trauma, etc.

Disbacteriosis (disbiosis) is a qualitative and quantitative misbalance of the normal flora. This can lead to an overgrowth of one or more of the pathogenic microorganisms (bacteria or fungi) which then may damage some of the other smaller beneficial ones.

Dysbiosis is the most prominent in the digestive tract or on the skin, but can also occur on any exposed surface or mucous membrane such as the vagina, lungs, mouth, nose, sinuses, ears, nails, or eyes.

It has been associated with the different illnesses, like inflammatory bowel disease and chronic fatigue syndrome.

Balance of normal flora is disturbed by such diverse things as repeated and inappropriate antibiotic exposure or alcohol misuse.

Treatment of disbiosis:

– **Probiotics** (lactulosa);

– **Eubiotics** (colibacterin, lactobacterin).

Lecture 6. ANTIMICROBIAL ACTIONS. CHEMOTHERAPY OF THE BACTERIAL INFECTIONS

MICROBIAL DECONTAMINATION is complete or partial removal of the microorganisms from the objects of surrounding environment or from the human organism with use of the factors causing direct damage of microorganisms.

Types of decontamination:

– Decontamination of the objects (sterilization and disinfection);

– Decontamination of the alive organisms (antiseptics and chemotherapy).

Antimicrobial agents:

Bactericides ► killing of microorganisms;

Bacteriostatics ► inhibition of bacterial multiplication.

STERILIZATION

Sterilization is a complete removal or killing of all microorganisms (vegetative cells and spores!) on the objects of environment. Objects after sterilization are called **sterile**.

Types of sterilization:

1. Sunlight (spontaneous).
2. Drying.
3. **Dry heat sterilization** (flaming, incineration, hot air).
4. **Moist heat sterilization** (pasteurization, boiling, autoclaving).

Autoclaving is a sterilization by hot water steam with use of high pressure; T of steam = 110–140°C, in autoclave).

Fractional sterilization (tyndallisation) is sterilization by the flowing steam (30 minutes under the temperature of 100°C, with several intervals for one day to cool the material and to enable the spores to germinate).

5. Filtration (candles, asbestos pads, membranes).
6. Radiation (gamma rays or UV rays).
7. Ultrasonic and sonic vibrations.
8. Chemical sterilisation: use formaldehyde, ethylene oxide.

DISINFECTION AND ANTISEPTIC

Disinfection is elimination or reduction of the definite group of the pathogenic microorganisms – usually with use of chemical agents (substances) which called **disinfectants.**

Disinfectants are bacteriocidal products used to kill the microbes on the inanimate objects or surfaces (may be sporostatic, but not sporocidal).

Antiseptic is inhibition of the growth (rare killing) of the disease-causing microbes on the intact and injured skin and mucous surfaces – usually with use of chemical agents which called **antiseptics**.

Antiseptics are chemical disinfectants which can be safely applied to skin or mucous membrane and are used for prevention infection by inhibiting the growth of the disease-causing bacteria.

Common disinfectants and antiseptics:

1. Ethanol (70-80%).
2. Formaldehyde, glutaraldehyde.
3. Phenol (carbolic acid).
4. Halogens (chlorine, iodine and their derivatives).
5. Metallic salts (salts of copper, mercury, $AgNO_3$).
6. Oxidants (ozone, H_2O_2, $KMnO_4$).
7. Surfactants (or detergents, or surface-active agents) included cationic, anionic, nonionic compounds.
8. Anilin dyes (brilliant green, crystal violet, malachite green).

ASEPSIS

Asepsis is creation of the zone free from any microorganisms in the next places: patient's areas, rooms where are medical manipulations, clinical laboratories (creation of the aseptic conditions).

Aseptic – characterized by the absence of the pathogenic microbes.

Septic – characterized by the presence of pathogenic microbes in the living tissues.

Methods of asepsis are sterilization, disinfection, antiseptics, and separation.

ANTIMICROBIAL AGENTS

1. **Antiseptics**: mercurials, silver nitrate, iodine solution, alcohols.
2. **Disinfectants**: chlorine, chlorine compounds, lye, copper sulfate, quaternary ammonium compounds.
3. **Preservatives**: static agents used to inhibit the growth of microorganisms, most often in foods.
4. **Chemotherapeutic agents**: antimicrobial agents of synthetic origin useful in the treatment of bacterial, viral diseases and tumors.
5. **Antibiotics**.

ANTIBIOTICS

Antibiotics are chemotherapeutic agents of natural, semisynthetic or synthetic origin that in low concentrations are able to cause inhibition or killing sensitive to them microorganisms or tumor cells inside of macroorganism.

Classification of antibiotics
according to groups of sensitive microorganisms:

1. Antibacterial.
2. Antiviral.
3. Antifungal.
4. Antitumoral.
5. Antiprotozoal.

Classification of antibiotics according to source of receiving:

- Fungi (Penicillium).
- Actinomycetes (80 % of antibiotics – Streptomyces).
- Bacteria (Bacillus and Pseudomonas).
- Synthetic antibiotics.

Classification of antibiotics according to their effect:

- Bacteriostatic;
- Bacteriocidal.

Classification of antibiotics according to spectrum of action:

1. Narrow spectrum.
2. Moderate spectrum.
3. Broad spectrum.

Classification of antibiotics according to mechanism of action on bacteria:

a) **Cell wall synthesis inhibitors**:

1. Beta-lactams (ex.: penicillins and cephalosporins).
2. Carbapenems.
3. Monobactams.
4. Glycopeptides.

b) **Cell membrane inhibitors:**

1. Polymyxin.
2. Nystatin.
3. Imidazole.

c) **Protein synthesis inhibitors:**

1. Tetracyclines (ex.: tetracycline, doxycycline).
2. Aminoglycosides (ex.: streptomycin, gentamycin).
3. Chloramphenicol (or laevomycetinum).
4. Macrolides (ex.: erythromycin).

d) **Nucleic acid synthesis inhibitors:**

1. Quinolones and fluorquinolones (ex.: nalidixic acid).
2. Rifamycines (ex.: rifampicin).

e) **Competitive inhibitors** (or anti-metabolites, growth factors analogs → they are specifically inhibiting essential metabolic pathways in the bacterial pathogen). Ex.: Sulfonilamides (trimethoprim or biseptol).

Principles of rational antibiotic therapy:

1. Microbiological principle.
2. Pharmacological principle.
3. Clinical principle.
4. Epidemiological principle.

Side effects of antibiotic therapy:

- Toxicity.
- Disbacteriosis.
- Depression of immunity.
- Allergy (anaphylactic shock).
- Mutagenic reactions on fetus (terratogenic effect).
- L-transformation.
- Development of antibiotic resistance.

Problem of drug resistance

Bacterial pathogen is able to develop or acquire resistance to antibiotic, then that substance becomes useless in the treatment of infectious disease caused by that pathogen.

Types of resistance:

- natural resistance;
- acquired resistance.

Problematic bacteria are bacteria which resistant to the antibiotics (→"problematic patients"), have often multiple resistance; produce the nosocomial (intrahospital) infections.

Examples of the most distributive **nosocomial agents:**

– enterobacteria;

– *pseudomonas aeruginosa*;

– staphylococci (MRSA – Methicillin Resistant *Staphylococcus aureus*);

– enterococci (VRE – Vancomycin Resistant Enterococci).

Antibiotics sensitivity (susceptibility) tests:

1. **Minimum inhibitory concentration tests:**

► broth dilution test;

► agar dilution test;

► method of E-tests.

2. **Disk diffusion test** (or method of the standard disks).

PART 2. INFECTOLOGY AND IMMUNOLOGY

Lecture 7. INFECTION. PATHOGENICITY OF THE BACTERIA. EPIDEMIOLOGY OF THE INFECTIOUS DISEASES

Infection is multiplication of infectious agent within the body.

Multiplication of a normal flora is generally not considered as infection (but at certain conditions normal flora produce opportunistic infections!); on other hand, multiplication of the pathogenic bacteria – even is person is asymptomatic – is deemed an infection.

Carrier is a person with the asymptomatic infection that can be transmitted to another person.

Parts of infection:

1. Microorganism.
2. Macroorganism.
3. Factors of environment.

INFECTIOUS DISEASE is rare, terminal consequence of the infection.

Infection may imply colonization, multiplication, invasion of a pathogen, but infectious disease is used to describe an infection that causes significant overt damage to the host.

Table 3 – Periods of infection disease:

Periods	Pathogen	Symptoms	Host is contagious?	Immune response
Incubatory	Adhesion	Without	No	No
Predromal	Colonization	Nonspecific	Probably	No
Clinical manifistations	Multiplication	Specific symptoms	Yes	Ig M then Ig G
Recovery – Complete – Incomplete (carrier state)	Death or slow multiplication	Normalization	Release during carrier state only	Ig G

PATHOGENICITY AND VIRULENCE

Pathogenicity is an ability of the infectious agent to cause a disease (it is genetic feature of the microbial species).

Classification of bacteria according to pathogenicity:

1. **Nonpathogen** is a microorganism that does not cause the disease;
2. **Opportunistic pathogen** (potential pathogen, facultative pathogen) is an agent capable of causing the disease only when host's resistance is impaired (patient is "immunocompromised");
3. **Pathogen (obligate pathogen)** is a microorganism capable cause the disease.

Opportunistic pathogens can cause the opportunistic infections or diseases only in certain conditions like:

– decrease of immunity;

– high infectious dose;

– change the site of localization due to penetrating trauma or medical manipulations (e.g., fecal flora enters urinary tract; skin flora binds with catheters and so on).

Virulence (degree of pathogenicity) is quantitative ability of an agent to cause disease (it is individual feature of the strain).

Virulence can be increased or decreased in vivo and in vitro (ex.: avirulent strain is strain which lack virulence).

Pathogenic factors (or virulence factors) are factors that are produced by a microorganism and evoke disease, includes:

1. Factors of adhesion and colonization.
2. Factors of invasion.
3. Toxins.

Adhesion (adherence, attachment) is process by which bacteria stick to the surface of host cells. Once bacteria have entered the body, *adhesion is initial step in the infectious process.*

Colonization is multiplication of bacteria on the body surfaces.

Factors of adhesion and colonization:

- Capsules.
- Adhesins (Pili/Fimbria) binding with receptors of host.
- LPS and Proteins of outer membrane (in Gram-negative bacteria).
- Teichoic acids (in Gram-positive bacteria).

Invasion is ability of pathogen to spread in the host tissues after establishing of infection.

Factors of invasion (invasions):

- **Special proteins** (internalin).
- **Different enzymes** (Hayluronidase, Neuraminidase, Fibrinolysin, Coagulase, also protease, nuclease, lipase, DNA-ase, RN-ase).

TOXIGENICITY (INTOXICATION) is ability of pathogen to produce toxins. Bacteria can have two types of toxins – **exotoxins** and **endotoxins**.

Classification of exotoxins due to affected cells:

- Neurotoxins (affect neurons).
- Cytotoxins (block of the protein synthesis in many different cells).
- Enterotoxins (affect enterocytes).
- Hemolysins (increase of membrane permeability and damage of RBCs).

Classification of exotoxins due to mechanism of action:

1. **AB-toxins** (binding subunit B and catalytic active subunit A);
2. **Membrane toxins** (formation of the pores in the different cells and cause their lysis, like hemolysins and leucocidins).

3. **Superantigenes** (stimulate macrophages and T-lymphocytes to produce excessive amounts of harmful cytokines).

Effects of Endotoxin:

1. Pyrogenicity (fever) or hypothermia.
2. Activation of host defense (complement activation, activation of macrophages and interferon synthesis).
3. Depression in blood pressure.
4. Endotoxic shock.

Table 4 – Characteristics of endotoxins and exotoxins

Property	Exotoxins	Endotoxins
1. Bacterial source	Gram-negative and Gram-positive bacteria	Only Gram negative bacteria
2. Location	Actively secreted by bacterial cells (extracellular)	Form part of the cell wall (outer membrane)
3. Composition	Protein	LPS
4. Stability	Heat labile	Heat stable
5. Antigenicity	Highly antigenic; stimulate formation the Ab-antitoxins	Weakly antigenic
6. Specificity	Specific effect for each exotoxin	Action common to all endotoxins
7. Enzymatic activity	Usually	No
8. Pyrogenicity	Occasionally	Yes
9. Interaction with specific antibody	Can be neutralized by antibodies	Neutralization by Ab is ineffective
10. Obtaining	Separation from bacterial culture by physical means as filtration	Obtained only by bacterial cell lysis
11. Form Toxoid (anatoxin)	Yes	No
12. Activity	Active in very minute doses	Active only in very large doses

Detection of virulence:

– *Direct revealing*: biological probe on animals.
– *Indirect revealing*: detection of enzymes of virulence in vitro.

Measurement of virulence:

- Dlm (Dosis letalis minima).
- LD 50.
- Dcl (Dosis certa letalis).
- ID (Infectious dose).

CLASSIFICATION OF INFECTIONS:

On the base of number of pathogens:

– Mono-infection
– Mixed infection

On origin:

– Exogenic
– Endogenic (Autoinfection)

On localization of pathogen:

– Localized
– Generalized (Bacteriemia, Sepsis, Virusemia, Endotoxemia, Toxenemia, Toxic shock)

Repeated infections:

– Re-infection
– Super-infection
– Secondary infection (opportunistic)
– Relapse
– Co-infection

On source of infection:

– Anthronoses
– Zoonoses
– Sapronoses

On spreading:

– Sporadic case
– Endemy
– Epidemy
– Pandemy

On place of beginning:

– Intra hospital (nosocomial)
– Out the hospital (natural)

On duration:

– Acute
– Subacute
– Chronic
– Fulminant and Slow

On clinical manifestations:

– Manifest
– Inapparent (subclinical)
– Atypical and Typical
– Severe and Mild

– Microbocarriage
– Latent
– Persistent

EPIDEMIOLOGY OF THE INFECTIOUS DISEASES

- **Fecalo-oral mechanism of transmission:**

Methods of transmission:

– Food-borne (alimentary).
– Water-borne.
– Contact (indirect).

Portal of entry – **GIT.**

- **Aerogenic mechanism (inhalation, air-borne):**

Methods of transmission:

– By droplets.
– By dust particles.

Portal of entry – **respiratory tract.**

• **Blood-borne mechanism:**

Methods of transmission:

– Transmissive (bites of vectors-insects).
– Parenteral (by inoculation, medical manipulations).
– Sexual.

Portal of entry – **blood.**

•**Contact mechanism:**

Methods of transmission:

– Contact through wounds.
– Direct contact.
– Indirect contact (with help of inanimate objects).
– Sexual contact (contact of mucous).

Portal of entry – **skin surfaces** and **mucous membranes** *(mainly injured).*

• **Congenital mechanism:**

Method of transmission:

– Transplacental.

Portal of entry – tissues of fetus.

Lecture 8. IMMUNOLOGY. NATURAL RESISTANCE AND IMMUNITY

Branches of immunology:

- general immunology;
- immunoprophylaxis (vaccinology);
- immunotherapy;
- immunodiagnostics;
- allergology;
- immunology of transplantation and malignancy;
- immunohematology;
- immunopharmacology.

IMMUNITY is a reaction of the body against any foreign material (ability to recognize and eliminate of genetically foreign antigen).

Classification of the types of immunity:

• On the base of mechanism:

– humoral (**AMI – antibody-mediated immunity**);

– cellular (**CMI – cell-mediated immunity**).

• On direction:

– antibacterial (antimicrobial);

– antitoxic;

– antiviral;

– antitumoral;

– autoimmunity.

• On base of origin:

– innate (inborn, natural, nonspecific);

– adaptive (acquired, specific).

• On participation of own immune system:

– active;

– passive.

Table 5 – Comparison between active and passive immunity

Active immunity	Passive immunity
Formation of own antibodies	Transferred by ready-made antibodies
Long-lasting (months and years)	Nonlong-lasting (several weeks)
Time is need for development of effect	Immediate effect

Innate (inborn) immunity is general protection against infections caused by the pathogens (1-st line of defense).

Levels of an innate immunity:

► **SYSTEM LEVEL (nonspecific resistance):**

• anatomical defenses;

• microbial antagonism (normal flora and bacteriocins);

• inflammation.

► **CELLULAR LEVEL (cellular nonspecific immune factors):**

• phagocytosis;

• null cells (NK-cells, K-cells).

► **HUMORAL LEVEL (humoral nonspecific immune factors):**

• tissue bactericidal substances.

Factors of nonspecific resistance:

1. Mechanical factors:

– intact skin and mucous (fatty acids, sweet, normal flora of skin);

– movement the secretions of the organs – urine, saliva, tears, sputum, feces and elimination of microbes with them;

– ciliated epithelium (also eliminates the microbes).

2. Chemical and biochemical factors:

– sweet (low pH and fatty acids);

– lysozyme is present in most biological liquids (tears, serum, saliva…) → break down of cell wall;

– rapid pH change from stomach to upper intestine;

– low pH of vagina.

3. Physiological factors like coughing, sneezing, vomiting, diarrhea, etc.

Inflammation is pathophysiological reaction of tissue to the pathogen that accumulates all antimicrobial factors in the site of infection.

Signs of inflammation: heat, swelling, pain, redness.

PHAGOCYTES

Professional phagocytes:

1. **Mononuclear phagocytes** (blood **monocytes** and tissue **macrophages**).

2. **Polymorphonuclear leucocytes (granulocytes) – microphages** (especially, neutrophiles are actively phagocytic and form the predominant cell type in acute inflammation, they are forming pus).

Monocytes leave circulation and reach varies tissues to become transformed into macrophages (alveolar macrophages in the lungs or Kupffer cells in the liver). Tissue macrophages proliferate locally and survive for months. Macrophages express many **surface receptors** (receptors for complement components, for immunoglobulins, CD14, and MHC class II).

Functions of macrophages:

1. **Phagocytosis** (primary function of macrophages).

2. **Antigen presentation** for T-cells (T-cells can recognize only processed and presented microbial Ag in connection with MHC).

3. **Secretary function** (they secrete a number of biologically active substances, including hydrolytic enzymes, tumor necrosis factor, colony stimulating factor, interleukine-1).

PHAGOCYTOSIS

Phagocytosis is intracellular killing of microbes by the phagocytic cells (intracellular nonspecific cytotoxicity).

Steps of phagocytosis:

1. **Delivery** of phagocytes to the site of an infection;

2. Phagocytic **adherence** (with help of opsonins like IgG, IgM, C3b and C5b).

OPSONIZATION – is coating of the particles (microbes) to improve adherence.

3. **Engulfment** of microbe and formation of phagocytic vacuole – phagosome.

4. **Metabolic stimulation** and **killing** of the microbes.

Mechanisms of killing:

► Oxigen-indipendent mechanism:

Fusion phagosome and lysosome and killing of a pathogen inside the lysosome by Lysozyme, Lactoferrin, Cationic Proteins, Hydrolytic Enzymes.

► Oxigen-dipendent mechanism:

• oxygen use is increased;

• accumulation of toxic products;

• “respiratory explosion” of microbes.

Then microbial antigens are inserted into the receptors on the surface of macrophage (MHC class II) for their **presentation** to T-lymphocytes to activate specific immune response.

NATURAL KILLERS (NK-cells)

• **Large granular lymphocytes** (LGL) that can lyses variety of cells including virus-infected cells and different tumor cells.

• Found in spleen, lymph nodes, bone marrow, blood.

• NK activity is “natural” or “nonimmune” as it does not require prior contact with Ag, their cytotoxicity is not Ab dependent and MHC restricted!

• They have CD16 and CD56 on their surface.

• They bind with glycoprotein receptors on the target cells and release several cytolytic factors. One of these, ***perforin*** causes transmembrane pores through which other cytotoxic factors enter the cell and destroy it by **apoptosis** (programmed cell death).

TISSUE BACTERIOCIDES:

• complement;

• lysozyme;

• peroxidase;

• interferons – IFN (antiviral proteins);
• ß-lysins (serum protein for lysis of CPM);
• interleukins (IL);
• acute phase protein (C-reactive protein – CRP);
• lactoferrin (iron-binding protein).

COMPLEMENT SYSTEM

Complement is a cascade of interacting proteins found in a serum.

• 20 proteins (main C1-C9, may be subunits **a** and **b**).

• Direction of cascade activation: **C1** → **C4** (C4a and C4b) → **C2** (C2a and C2b) → **C3** (C3a and C3b) → **C5** (C5a and C5b) → **MAC**.

• Terminal step of the activation is formation of **membrane-attack complex** (MAC) consisting of C5b-C9 ► put the holes in membranes of a microbe ►enter of water and electrolytes ► osmotic lysis of a microbe.

• It has two pathways of activation:

1) **classical pathway** (initiated by Ag-Ab complex);

2) **alternative pathway** (initiated by bacterial LPS).

Functions of complement system

1. Increase rate of inflammation (C3a, C4a, C5a are called **anaphylotoxins**).

2. Opsonization of microbes (C3b, C5b are called **opsonins**).

3. Damage of pathogens (formation of **MAC** which causes microbial lysis).

Lecture 9. IMMUNE SYSTEM OF HUMAN BODY. IMMUNOLOGICAL METHOD OF DIAGNOSIS OF THE INFECTIOUS DISEASES

Immune system:

• System for protection against infecting and other foreign agents (antigens – Ag) by distinguishing self from non-self.

• Provide the immunity – CMI or AMI.

Functions:

1. Defense from "not-self";
2. Elimination of modified "self“(cancer cells);
3. Regulation of cells growth;
4. Regulation the homeostasis of human organism.

Functional organization of the immune system:

• organic level;

• cellular level;

• molecular level.

ORGANIC LEVEL

1. **Primary (central) organs**:

• Thymus ►T-cells maturation (T-lymphopoiesis) .

• Bone marrow ►All the immune cells are born and only B-cells mature (B-lymphopoiesis).

2. **Secondary (peripheral) organs**:

• Provide specific immune response (interaction of B and T-cell with Ag).

• Lymph nodes and spleen.

• Mucous associated lymphoid tissue (MALT) including tonsils, Peyer's patches, follicles, appendix and others.

• Skin associated lymphoid tissue (SALT).

• Gut associated lymphoid tissue (GALT).

CELLULAR LEVEL

1. **Antigen presenting cells** – APCs (macrophages, dendritic cells, B-cells).

2. **Regulatory cells** (T-helpers, T-suppressors).

3. **Effector cells** (B-cells, CTLs, neutrophiles, macrophages, NK-cells, mast cells); **CTL - Cytotoxic T-lymphocytes** (outdated name - T-killers).

4. **Memory cells** (memory T and B-cells).

ANTIGEN PRESENTING CELLS

1. **Macrophages** are major antigen presenting cells.

2. **Dendritic cells** also perform this function. They possess MHC class II but have no phagocytic activity! They are specifically involved in the presentation of Ag to T-cells during the primary immune response.

3. **B-cells** are involved particularly during the secondary immune response.

4. **Langerhans cells** in the skin possess features of macrophages and dendritic cells, they process and present antigens that reach the dermis.

Immunocompetent cells are cells which participate in the development of immune response (T- and B-cells).

B-cells are cells responsible for antibody-mediated immune response and provide the production of antibodies.

T-cells are cells responsible for induction of immune response. There are two subpopulations of T-helpers: Th1 and Th2. They produce different cytokines (see below) and activate different types of immunity.

• **Th1** produce **IL-2, gamma IFN** and **lymphotoxin** → activation of macrophages, NK, neutrophiles, CTLs (provide CMI).

• **Th2** produce **IL-2, 4, 5, 6, 10, 13** → activation of B-cells, mast cells and eosinophiles **(**provide AMI).

Table 6 – Characteristics that distinguish T from B-lymphocytes

Characteristic	T-cells	B-cells
Cell type	Mononuclear leukocyte (lymphocyte)	Mononuclear leukocyte (lymphocyte)
Ag binding receptor	**T cell receptor** with co-receptor (TCR/CD3) TCR recognize protein Ag with help of MHC!	**B cell receptor** with co-receptor (BCR/CD79a/b) BCR can recognize soluble Ag of any chemical composition (without MHC help)!
Surface markers	CD3, CD4, CD4	CD 19, CD20, CD21, CD40
Chief secretary products	Lymphokines	Antibodies

MOLECULAR LEVEL (Ig super family):

1. **BCR** – **B**-**c**ell **r**eceptor for Ag recognization (structure of BCR: membrane Ig M and Ig D and co-receptor CD79 a/b);

2. **TCR** – **T**-**c**ell **r**eceptor for Ag recognization (structure of TCR: Alpha + beta or delta + gamma globulin chains with constant (C) and variable (V) regions and co-receptor – CD3);

3. **Soluble immunoglobulins** (IgG, IgM, IgD, IgA, IgM);

4. **Antigen-presenting molecules**: human leukocyte antigens (HLA) or major histocompatibility complex (MHC);

5. **Cytokines;**

6. **Adhesins;**

7. **CD** molecules ("cluster of differentiation" or cluster designation markers) that are expressed on the cell membranes and provide the information about specify of the cells, their origin, stage of activation and differentiation. Each marker has been given CD number such as CD1, CD2,

CD3, etc. For example, it is known that HIV infects and destroys the CD4+ T-cells, also known as T-helpers.

Cytokines

• Substance secreted by any type of leukocytes in a response to Ag.

Types of immunocytokines:

• **Interleukins** (IL) ► cytokines produced by one class of the leukocytes for activating of other class.

• **Colony-stimulating factor** (CSF).

• **Interferon** (IFN).

• **Tumour necrosis factor** (TNF).

• **Chemokines.**

• **Monokines.**

• **Lymphokines.**

IMMUNOLOGICAL METHOD OF DIAGNOSIS

Immunological (serological) method is a method based on the specific interaction between antigens and antibodies.

Aims of the serological method:

1. Serological diagnosis of the infection disease which is based on detection of unknown Ab in serum of patient with help of known Ag in diagnosticums is called **serodiagnostics**.

Principles of the giving during serodiagnostics:

• diagnostic titer for the certain disease;

• increase titer in the paired sera of the patient in 4 or more times;

• detection of classes of Ig: IgM is sign for the acute infection and IgG is sign for the chronic infection or immunity.

2. Serological diagnosis of the infection diseases which is based on detection of unknown microbial Ag in the biological liquids or tissues of the patient with help of known Ab in diagnostic antisera is called **express-diagnostics**.

3. Serological identification of the unknown Ag of pure culture (detection of the serotypes of the pure culture) with help of known Ab in diagnostic antisera is called **serotyping.**

Diagnosticum is known killed microbes or Ag (O-diagnosticum, H-diagnosticum, erythrocytic diagnosticum, viral diagnosticum, etc.)

Diagnostic antiserum is a serum containing specific Ab of known specificity and titer (monovalent serum, polyvalent serum, agglutinating serum, precipitating serum, antitoxic serum, antiglobulin serum, etc.).

Serological method includes serological reactions (agglutination, precipitation, neutralization, complement activation, IFA, ELISA and others).

SEROLOGICAL REACTIONS are reactions between Ag and Ab on the basis their specificity to each other and formation of immune complexes (IC).

DOUBLE SEROLOGICAL REACTIONS

1. **REACTION OF AGGLUTINATION** (RA):

• Sticking of particulate Ag (agglutinogen: bacteria, erythrocytes) with specific Ab (agglutinins) in the presence of an electrolyte.

• Ag and Ab react must be specific to each other!.

• Positive result – formation of immune complexes - agglutinate ("small white grains" in the transparent drop of a serum).

• RA classification: **direct** (may be slide or tube agglutination) and **indirect/passive** (may be latex-agglutination - RLA, co-agglutination - RCA, and indirect hemagglutination – RIHA).

2. **REACTION OF PRECIPITATION** (RP):

• Sedimentation of soluble Ag (precipitinogen: exotoxin, drug) with specific Ab (precipitinins) in presence of an electrolyte;

• Ag and Ab react must be specific to each other!

• Positive result – formation of insoluble immune complexes - precipitate (appearance of turbidity in liquid or lines (bands) of precipitation in gel);

• RP classification: in liquid phase - Tube Precipitation, Flocculation; in gel – Immunodiffusion, Immunoelectrophoresis.

Precipitation in a gel is called **immunodiffusion.**

Types of immunodiffusion:

1. Radial immunodiffusion.
2. Single diffusion.
3. Double diffusion (Ouchterlony test).

3. **REACTION NEUTRALIZATION** (RN):

• Neutralization of effects of exotoxins or viruses by specific antibodies.

Types of neutralization in vivo:

1. Skin tests for estimation antitoxic immunity (Dick test, Shick test):
2. RN of toxins or viruses on animals.

Also flocculation and Ouchterlony test for *C.diphtheriae* can be considered as neutralization tests (RP+RN).

COMPLEX SEROLOGICAL REACTIONS

COMPLEMENT FIXATION TEST (CFT):

• Ability of Ag-Ab complex to "fix" complement.

• Indicator system is **hemolytic system** (consists of erythrocytes of sheep and hemolytic serum).

• Source of a complement is guinea pig serum or dry complement.

• Positive CFT is indicated by the negative hemolysis, negative CFT is indicated by the positive hemolysis.

LABELED REACTIONS

1. IMMUNOFLUORESCENCE REACTION (IFA):

• Principle: “Labeling” (marking) of Ab or anti-Ab with fluorescent dye which converts ultraviolet rays into visible light (use luminescent microscopy for detection of the results).

• Positive IFA: green shining of microbes (Ag) on the dark background.

• IFA may be direct and indirect.

2. **ENZYME LINKED IMMUNOSORBENT ASSAY** (ELISA):

• Principle: “Labeling” (marking) of Ab or anti-Ab with enzyme (peroxidase) which decompose substrate and it changes color due to chromogen (use photometry for detection of the results).

• Positive ELISA: colorless substrate becomes substrate of yellow color.

• Known Ag or Ab are adsorbed on the solid phase.

• ELISA may be direct and indirect.

3. **IMMUNOBLOTTING** (Western blot):

• Principle: electrophoresis + ELISA (or RIA).

• Whole microbes are decomposed into certain Ag with help of electric force in gel according to their molecular mass (electrophoresis).

• Then these Ag or Ab are adsorbed on the cellulose paper (“blots”) and other steps of reaction are carried out as ELISA.

• Positive result: appearance dark bands on the cellulose paper.

• Can be detected certain Ag or Ab against certain Ag!

4. **RADIOIMMUNE ASSAY** (RIA):

Principle: “Labeling” of Ab or Ag with **radioactive isotope** and detection of the results with help of radioactive counters.

Lecture 10. ANTIGENS. IMMUNE RESPONSE. CELL-MEDIATED IMMUNE RESPONSE (CMI)

Antigen is certain kinds of substances foreign to the host and able to trigger the immune response in the host (formation of Ab and binding with them, activation of immune competent cells and binding with their receptors).

Antigens are large molecules which have specific chemical groups – **antigenic determinants**, or **epitopes** (will react with paratopes of antibodies).

Attributes of antigenicity:

• **IMMUNOLOGICAL REACTIVITY** is specific reaction of Ag with Ab or the immune cells.

• **IMMUNOGENICITY** is an ability of Ag to induce the immune response.

Types of Ag:

1. **Complete Ag** or **immunogens** (induce Ab formation and can bind with Ab so produced). Ex: bacterial and viral Ag.

2. **Haptens** or incomplete Ag (incapable to induce Ab formation but can react specifically with Ab). Become immunogenic after combining with a protein molecule - carrier. Ex: low-molecular weight compound like antibiotics.

Classification of Ag:

• **on origin:** exogenic and endogenic Ag;

• **on composition:** protein and non-proteins;

• **on participation of T-helpers** in activation of B-cells: T-dependent and T-independent Ag;

• **on immunogenicity:** haptens and immunogens;

• **on number of epitopes**: monovalent and polyvalent.

Determinants of antigenicity:

1. Size of Ag.
2. Chemical nature of Ag.
3. Susceptibility of Ag to tissue enzymes.
4. Degree of foreignness of Ag.

Most of good Ag is macromolecules such as proteins and polysaccharides, lipids or nucleic acids are rare antigenic. Substances of low molecular weight do not make good antigens.

Antigenic specificity:

– species, organ and tissue specificity (organ Ag);

– isospecificity (erythrocyte Ag and MHC/HLA Ag);

– autospecificity. Ag usually foreign to the host and recognized as such by the immune system, if this was not so, individual may respond immunologically against his or her own body constituents (it is autoantigens), with the potential of producing of tissue damage (autoimmune diseases).

Bacterial Ag:

- group Ag (for some species);
- specific Ag (for species);
- typical Ag (for certain serotype);
- capsular Ag (K-Ag);
- cell wall Ag (O-Ag);
- flagella Ag (H-Ag);
- ribosomal Ag;
- Ag of toxins, enzymes.

Protective Ag (highly immunogenic and provide development of the protective level of Ab in immunity).

SYPERANTIGENS are Ag which induce non-specific multiple activation of T-lymphocytes and after activation lead to its apoptosis. Ex.: enterotoxins, cholerotoxin, staphyloccocal toxin, some viruses.

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

MHC is collection of highly polymorphic genes encoding the proteins which regulate immune response.

Antigens of MHC are surface glycoproteins of the different cells which responsible for **immune recognization** of CD receptors during Ag presentation for T-cells (they help T-cells recognize and bind Ag!).

May be MHC class I and MHC class II (also MHC class III that encode complement molecules and cytokines).

In humans, MHC is called **HLA** (their corresponding genes are found on the short arm of chromosome 6).

Table 7 – Characteristic features of different MHC classes

Feature	MHC I	MHC II
Localization	All nucleated cells and platelets	Antigen-presenting cells (macrophages, B-cells, dendritic cells)
Types	HLA-A, HLA-B, HLA-C	HLA-DP, HLA-DQ, HLA-DR
Function	Ag recognition by T killers (bind with CD8)	Ag recognition by T helpers (bind with CD4)
Structure	Alpha heavy chain (3 domains – α1, α2, α3, light β2-microglobulin)	Alpha (α1 and α2) and beta chains (β1 and β1)

Importance of MHC:

- Immunological recognization of Ag by the immune competent cells for development of immune response (antigen presentation).
- Immunogenetical and biological individuality of every organism (marker of “self” on every cell).
- Importance in compatibility of transplanted tissues.

ANTIGEN PRESENTATION

1. **Presentation of Ag for activation T helpers**:

Base of presentation is interaction between macrophages and T-helpers.

► Ag is recognized by TCR of T-helper;

► MHC II class is recognized by CD4 receptor of T-helper.

2. **Presentation of Ag for activation CTLs**:

Base of presentation is interaction between T-killers (CTL) and target cells.

► Ag is recognized by TCR of T-killer;

► MHC I class is recognized by CD8 of T-killer.

IMMUNE RESPONSE

Immune response is specific reactivity induced in a host by antigen. In infectious disease is known it is generally equated with protection against pathogen ("immunity"). But the immune response has a much wider scope and includes reactions against any antigen, living (bacteria, fungi) or nonliving (pollen, food). Result of immune response may be beneficial, indifferent or injurious to the host! The immune response can be two types – AMI and CMI. The two are usually developed together, though at times one or the other may be predominant or exclusive. They usually act in connection but may sometimes act in opposition.

AMI provides:

- defense against most of the pathogens with predominant extracellular localization (some bacteria);
- primary defense against viruses that infect respiratory or intestinal tracts;
- participation in the pathogenesis of immediate type of hypersensitivity and certain autoimmune diseases;
- formation of antitoxic immunity (against exotoxins of bacteria).

CMI provides:

- protection against fungi, protozoa and viruses;
- protection against obligate and facultative intracellular bacterial pathogens;
- participation in rejection of transplanted tissues and organs;
- participation in the pathogenesis of delayed type of hypersensitivity and certain autoimmune diseases;
- formation of antitumoral immunity.

CELL-MEDIATED IMMUNE RESPONSE (CMI)

Type of immunity that is mediated by specific effector T-cells and other different cytotoxic cells (macrophages, NK-cells, etc).

Cells of CMI:

1. **APCs** – macrophages and dendritic cells;

2. **Effector T-cells**;

- T_{DTH} ► delayed type of hypersensitivity.
- CTLs ► specific extracellular cytotoxicity.
- NK-cells ► natural extracellular cytotoxicity.
- K-cells ► ADCC (antibody dependent cell-mediated cytotoxicity).
- Macrophages ► intracellular nonspecific cytotoxicity (phagocytosis).

3. **Regulatory T-cells –** T-helpers and T-suppressors (Th-1 is responsible for induction CMI, they produce cytokines: *IL-2, gamma-IFN* and *TNF (lymphotoxin)* → activation of macrophages, neutrophiles, CTLs).

4. **Memory T-cells** (secondary immune response).

Main stages of CMI:

For example, during *viral infection*, viral epitopes are presented on the surface of the virus infected cell bound to MHC class I.

1. In this form viral epitopes are recognized by **cytotoxic T-cells** (CTLs).

Mechanism of killing: CTLs during contact with target cell release the cytolytic molecules (**perforins** and **granzymes** → **target cell apoptosis**).

2. In order for expansion of the particular clone of cytotoxic T-cells to occur, **T-helpers** must also be activated. This occurs by phagocytosis of the virus by macrophages. Then viral epitopes are presented on the cell surface of macrophage together with MHC class II. T helpers recognize viral epitopes together with MHC class II and activate.

3. Activated T helpers (Th1) produce **cytokines** (IL-2, gamma-IFN, TNF) that stimulate differentiation and proliferation of both T-helpers themselves and cytotoxic T-cells.

4. Additionally **different cytotoxic cells** (macrophages, NK-cells, K-cells) are activating and realizing of their cytotoxicity on the target cells.

5. **T_{DTH}** (type of Th) participate in pathogenesis of DTH (if antigen is allergen for immune system).

6. **T memory cells** are also forming; they remain fallowing an infection and are ready to develop immune response more rapidly.

Lecture 11. IMMUNOGLOBULINS. ANTIBODY-MEDIATED IMMUNE RESPONSE (AMI)

ANTIBODIES (IMMUNOGLOBULINS)

Antibodies belong to group of proteins known as **globulins.** Since these globulins are involved in the immune response, they are known as **immunoglobulins (Ig).**

Antibodies are proteins produced in response to Ag.

In constitute 20-25% of the total serum proteins. There are five classes of Ig have been recognized – IgG, IgA, IgE, IgM and IgD.

Clonal selection theory

Although many millions of B cells are present in the body, only a small number are capable to respond to any one antigen! Clonal selection theory explains haw it works. When Ab is first produced by B cells, they are not secreted but are inserted into the cell membrane of B cell. When Ag comes into contact with B cell that has the corresponding Ab that B cell is triggered to divide and mature into a clone of plasma cells, *all produced Ab with the same specificity*. This clonal expansion accounts for increase in the number of the particular type of plasma cells.

Basic structure of Ig molecule

• The molecule is Y-shaped and consists of two heavy chains (**H-chain**) and two light chains (**L-chain**).

• Each of chain, in turn, consists of **variable region** and **constant region**. Constant regions for all molecules of the same Ig have the same amino acids. On other hand, variable region is the section of the molecule that interacts with the antigenic epitopes. Since each Ag is different from another, the variable regions of Ab are also different in their arrangement of amino acids.

• In the presence of papain (enzyme) Ig is spited into three fragments - one insoluble fragment which **c**rystallized in the cold (called **Fc-fragment**), and two identical soluble fractions which can bind with antigens (called **f**ragment **a**ntigen **b**inding – **Fab**).

• H-chains are distinct for each class and are designated by the Greek letter (IgG – γ/gamma/ H chain, IgA – α/alpha/, IgM – μ/mu/, IgE – ε/epsilon/, IgD – δ/delta/). L-chains are similar in all classes of Ig. Ig can have either κ/kappa/ or λ/lambda/, but never both.

CHARACTERISTICS OF EACH CLASS OF IMMUNOGLOBULINS

IgG

- Monomer (bivalent).
- 75-80% in serum (8-16 mg/ml).
- Activation of complement.
- Neutralization of toxins and viruses.

• Opsonization during a phagocytosis.
• Transport across the placenta → passive immunity of newborn.
• ADCC (bind with K-cells).
• Late Ab (indicates about chronic infection or immunity after infection).

IgA

• 10-15 % in serum (0.6-4 mg/ml);
• Two forms:
1. Serum IgA (monomer).
2. Secretary IgA/sIgA (dimer or trimer) presents in secretions (tears, saliva, brest milk) and mucous and provide local immunity against respiratory and intestinal pathogens.
• Complement activation.

IgM

• 8-10% in serum (0.5-2 mg/ml);
• pentamer (valency 10);
• first Ig synthesized by fetus;
• first Ig which appears after Ag contact (indicates about acute infection);
• activation of complement;
• component of BCR (with IgD).

IgE

• monomer (monovalent);
• less than 0.003-0.5 % (few mg/ml);
• ability to bind with mast cells → immediate type of hypersensitivity;
• high level indicates about helminthic infection.

IgD

• monomer;
• less than 0.3-0.5 %;
• component of BCR (with IgM).

Generally:

• IgG: protection body fluids (►secondary immune response);
• IgM: protection of bloodstream (►primary immune response);
• sIgA: protection body surfaces and mucous membranes (►local immunity);
• IgE: immediate type of hypersensitivity;
• IgD: BCR (recognization of Ag).

Classification of Ab (Ig)

• **Natural Ab** (present in blood serum without activation by Ag).
• **Immune Ab** (accumulate in a serum after introduction of Ag).
• **Circulating molecules**: all serum Ig and sIgA.
• Components of **BCR** (IgM and IgD).

• **Complete Ab** → all Fab-components work best and can cause visible by eyes reaction of an agglutination.

• **Incomplete Ab** → can't cause visible reaction of agglutination.

Functions of Ab (Ig)

1. Opsonization (IgG, IgM, C3b are best opsonins).
2. Complement activation:
• IgG and IgM → classical pathway;
• Ig A → alternative pathway.
3. Antibody-dependent cell-mediated cytotoxicity (ADCC).
4. Allergy (IgE).
5. Toxic neutralization (Ab-antitoxins).
6. Agglutination and precipitation reactions.
7. Effect on microbial physiology.

ANTIBODY-MEDIATED IMMUNE RESPONSE (AMI)

Type of immunity that is mediated by soluble host proteins called antibodies or immunoglobulins (it is also called **humoral immunity**).

There are two types of antigens which can induce AMI: **T-dependent antigens** (complex Ag like proteins and erythrocytes) and **T-independent antigens** (polysaccharides and other structurally simple molecules with repeating epitopes).

T-dependent activation of B-cells:

• Participation of T-helpers.
• All Ig are produced.
• B memory cells are present.

T-independent activation of B-cells:

• Direct stimulation B-cells without T-helpers.
• Only IgM are produced.
• B memory cells are absent.

Cells of AMI:

1. **APCs –** macrophages and dendritic cells;
2. **Effector cells** – B-cells, plasma cells;
3. **Regulatory cells** – T-helpers cells (Th2) which produce IL-2, 4, 5, 6, 10, 13.
4. **Memory B-cells** (secondary immune response).

B-cells come from stem cells of bone marrow, upon leaving of bone marrow B cells are carried out to the lymph nodes, Peyer's patches, tonsils and other peripheral immune organs. When B-cells interact with a foreign antigen, they are stimulated to differentiate and mature into the plasma cells.

Plasma cells (antibody-producing cells) are highly specialized for producing and secreting large amounts of antibodies.

Main stages of AMI (with T-dependent Ag):

1. Processing and presentation of Ag by **APC** bound with MHC class II to **T helpers** for recognization. B-cells, which possess BCR and MHC class II, can also present antigens to T-helpers, particularly during the secondary immune response.

2. **Activated Th2 cells** form IL-2 and other cytokines (IL-4, 5, 6, 10, 13) required for B-cells stimulation.

3. **B-cells** which have combined with their Ag proliferate (formation of a clone) and differentiate into the plasma cells and B memory cells.

4. **Plasma cells** begin to produce antibodies against antigen.

5. **B memory cells** are also forming; they remain fallowing an infection and are ready to develop immune response more rapidly.

Effector mechanisms of Ab (for elimination of Ag):

1. Immune phagocytosis (opsonization).

2. Complement-dependent lysis.

3. ADCC.

4. Neutralization of toxins and virus-infected cells.

Primary immune response is developing after first contact with antigen.

Characteristics:

• Slow in onset (latent period 3-5 days before Ab become detectable and within 15-30 days reach effective concentration for protection).

• Low concentrations of Ig are forming.

• “Short lived” (after short time – 1-6 months - Ab level declines).

• IgM are produced only.

Secondary immune response – after repeated contacts with same antigen.

Characteristics:

• Rapid in onset (several hours or 1-2 days).

• High concentrations of Ig are forming.

• “Long lived” (Ab remain detectable for months or years).

• IgG are produced mainly (in allergy – IgE, locally - sIgA).

Monoclonal Ab are Ab produced by the clone of B cells; such Ab have of the same specificity (can react only with specific and identical Ag).

Lecture 12. ALLERGY (HYPERSENSITIVITY). IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY OF THE INFECTIOUS DISEASES

DEFINITION ABOUT ALLERGY

ALLERGY (hypersensitivity to Ag) is a super efficient immune response. Antigen which causes allergic reaction is called **allergen**.

Classification of allergens: **exoallergens** (infectious and noninfectious) and **endoallergens** (extra barrier tissues antigens, autoallergens).

After first contact with allergen **sensitization** is developed (immune cells are activated and immunoglobulins are sensitized), but only *after second (repeated) contact with the same allergen* **clinical symptoms** of allergy will be appeared and damage of the tissues and organs is present.

CLASSIFICATION OF ALLERGIC REACTIONS

1. Immediate type of hypersensitivity (ITH) is called so because it is developing within 20-30 minutes after contact with the allergen and includes several subtypes:

1 type – **Actually immediate hypersensitivity (anaphylaxis);**

2 type – **Cytotoxic hypersensitivity;**

3 type – **Immune complex hypersensitivity;**

All of these subtypes are antibody-mediated (effector cells are B cells).

2. Delayed type of hypersensitivity (DTH) that gets its name from the long time (48-96 h.) that it takes for a skin reaction to Ag. It is cell-mediated type of allergy (effector cells are macrophages, T effector of DTH, T-helpers).

Actually immediate hypersensitivity

• First contact with allergen → activation of B-cells → production of IgE → Ig E is binding with basophiles and mast cells → mast cells are sensitized and laying in wait for second contact with allergen;

• Second contact with allergen → allergen interacts with IgE which connected with mast cells → degranulation of mast cells → release of mediators of inflammation (histamine, heparin, prostaglandin, etc) → physiological response (smooth muscles contraction, increase of vascular permeability, mucous secretion, etc).

Types of the clinical forms of anaphylactic hypersensitivity:

1. Localized anaphylaxis (atopic allergy).

• Allergens: pollen, fungal spores, dust mites, suspected food.

• Examples: *Hay fever (allergic rhinitis), allergic conjunctivitis, bronchial asthma, true food allergy, urticaria (hives).*

2. Systemic anaphylaxis (generalized response).

• Allergens: penicillin, antisera, venom of bees and wasps.

• Examples: *Anaphylactic shock.*

ATOPY is hereditary predisposition to hyper production of IgE to allergens.

Cytotoxic hypersensitivity

• First contact with allergen → activation of B-cells → production of IgM and IgG → binding of IgM and IgG with different cells of body (mainly: blood cells) → cells are sensitized;

• Second contact with allergen → production of organ (tissue)-specific Ab.

• Mechanisms of destruction of the tissues:

1. Complement-mediated cytotoxicity;

2. ADCC (by K-cells).

• Examples of the clinical forms: *drug allergy; hemolytic disease of newborn, autoimmune hemolytic anemia, leucopenia, thrombocytopenia, autoimmune diseases of the different organs.*

Immune complex hypersensitivity

• Involves Ab (IgM and IgG) that react with free antigens → **circulating immune complexes** (CIC) → deposition of CIC in organs and tissues (most common kidney, skin, blood vessels, and joints) → activation of complement → tissue damage and inflammation.

• Examples of the clinical forms: *Arthus reaction, serum sickness, pneumonitis, rheumatoid arthritis, glomerulonephritis, SLE (systemic lupus erythematosus).*

Delayed hypersensitivity (cell-mediated hypersensitivity)

• T-cell-mediated immune response to allergens.

• Allergens of DTH:

1. **Infectious**: intracellular bacterial pathogens (Mycobacteria); viruses, fungi, protozoa ► chronic infections with DTH in pathogenesis (infectious allergy).

2. **Non-infectious**: poison ivy, nickel, formaldehyde, latex, dyes in clothing and cosmetics (they are haptens).

Examples of the clinical forms: *skin tests (like Tuberculin test), certain chronic bacterial infections (TB, leprosy), contact dermatitis.*

Laboratory diagnosis of allergic reactions

• 1 type: skin tests (registration within 20 min) – revealing of IgE.

• 2 type – Revealing of Ag to blood cells.

• 3 type – Revealing of CIC.

• 4 type - Skin allergic tests.

IMMUNOPROPHYLAXIS (IMMUNIZATION)

Prevention of the infectious diseases by the creation of different types of the artificial immunity in the human body.

Forms of immunoprophylaxis:

• **Routine immunization** (schedules have been developed for different countries → "Vaccine preventable diseases": diphtheria, pertussis, tetanus, polio, measles, mumps, rubella, tuberculosis).

Bacterial vaccines in childhood immunization schedule:

• DPT ►diphtheria, pertussis (whooping cough), tetanus;
• DTaP ►… acellular pertussis…
• Hib ►Haemophilus influenzae type b;
• PCV ►pneumococcal vaccine;
• BCG ►Tuberculosis (TB);
• OPV ►oral polio vaccine (Sabin vaccine);
• IPV (IVP) ►inactivated polio vaccine (Salk vaccine);
• MMR ► measles (rubeola), mumps and rubella (German measles);
• HBV ►hepatitis B vaccine;
• Varicella ►varicella (chicken pox) vaccine.

• **Individual immunization** (typhoid vaccine, varicella vaccine, hepatitis B vaccine, cholera vaccine). Can be used in the cases:

– In endemic regions;
– after suspected bites;
– for laboratory and medical workers;
– individual predisposition.

Types of immunoprophylaxis:

1. Active immunoprophylaxis (used vaccines → *active artificial immunity*).

2. Passive immunoprophylaxis (used prophylactic antisera → *passive artificial immunity).*

VACCINES

Vaccine is a preparation which contains the microbes (bacteria, viruses) or their components (pure Ag, toxin, genes, ribosomes) and capable to produce the **active artificial immunity.** Application of vaccines: immune prophylaxis (**vaccination**) and treatment (**vaccinotherapy**).

Effective vaccine:

• must be safe;
• provides long-term protection;
• must be immunogenic (can induce high level of Ab formation);
• must be biologically stable (areactive);
• cheap to produce;
• easy to administration.

Types of the vaccines:

• Monovaccines (1 species).

• Combined vaccines (DPT, MMR);

• Monovalent, trivalent, heptavalent (number of serotypes).

• Conjugate (example: polysaccharide Ag + proteins (tetanus toxoid).

Classification of the vaccines:

1.**Live attenuated vaccine** (bacterial and viral).

2. **Inactivated (killed) vaccine.**

3. New generation of killed vaccines — **Fractional vaccines** (consist of purified microbial subunits):

— subvirion vaccine;

— surface Ag (protective Ag);

— toxoid (anatoxin);

— capsular polysaccharides;

— recombinant-vectors vaccines (genetically-engineered Ag: genes + vectors).

5. **DNA-vaccines** (viral gene + plasmid + promoter).

6. **Anti-idiotype-specific monoclonal Ab.**

Characteristics of the live vaccines:

• Attenuated (weakened) form of the "wild" virus or a bacterium.

1. Immune response similar to the natural infection - long-lived immunity.

2. Usually produce immunity with one dose, except those administered orally.

3. Severe reactions possible (allergy, disease, convulsions, high fever) ►*Do not use in immunocompromised persons and during pregnancy!*

4. Fragile — must be stored and handled carefully.

Characteristics of the killed vaccines:

• Produced by inactivating of the microbes.

• In the case of fractional vaccines, microbe is further treated to purify only those components to be included in the vaccine (e. g., the polysaccharide capsule of pneumococcus).

1. Less immunogenic but more safe.

2. Generally require 3-5 doses (booster doses).

3. Immune response mostly humoral.

4. Ab titer diminishes with time.

5. Need addition of “adjuvants” ($AlPO_3$) – for toxoids.

6. May be administered parenterally.

Methods of inactivation:

• Killed vaccines contain the microbes which inactivated by heating, UV, chemicals but not denaturated!

• Toxoid (anatoxin) – inactivated exotoxin by heating, it is not virulent but stay immunogenic!

Booster shots

Repeated inoculations of vaccines to maintain a high titer (level) of Ab.

Example: *epidemics in school may prompt recommendations for specific boosters.*

Complications of vaccination:

– autoimmune reactions;
– development of disease in immunosuppressed individuals;
– contra-indications for vaccination (cancers, pregnancy);
– hypersensitivity.

PASSIVE IMMUNOPROPHYLAXIS

Transfer of preformed Ab from the donor (animal or human) to the recipient (immunity is short-lived but fast). Routinely administrated to *botulism, tetanus, diphtheria, hepatitis, measles and rabies.*

Preparations for passive immunization:

1. **Antisera** (hyperimmune serum).
2. **Pooled human Ab** (immune globulin - Ig).
3. **Hyperimmune globulin** ((Ab against specific Ag).

Preparation which contains Ab is called immune serum or antiserum.

Classification of antisera

1. **Heterologous antisera** (hyper immunization of animals/horses by Ag).
2. **Homologous antisera** (from human donors convalescing from natural infections or donors artificially immunized).

Types of antisera

– antibacterial (rare use);
– antitoxic;
– antiviral;
– anti Rh+ – Ig (D);
– anti venom (snake, bees) – Antivenins.

Immunoglobulins

Ab-containing solution derived from human blood serum.

Obtaining – cold ethanol fractionation of large pools of a plasma.

May be intravenous and intramuscular preparations. Receiving from human donors that had been suffered from certain infections or after active immunization; also from post-abortion tissues.

Types of Ig:

• Pooled human Ig;
• Hyperimmune Ig.

Examples of antisera and Ig wide used in practical medicine:

– diphtheria antitoxin;
– tetanus Ig;
– hepatitis B Ig;
– botulism equine antitoxin;
– rabies antitoxin.

Application of antisera and Ig:

1. Postexposure and preexposure prophylaxis (passive immunization).
2. Immunotherapy:

– use for immunotherapy of the chronic infections or in emergency for prevention and treatment of certain diseases (tetanus, tick-born encephalitis, diphtheria, botulism);
– treatment of immunodeficiencies.

IMMUNOTHERAPY

Influence on immune system with the purpose of stopping of the pathological process.

Preparations for immunotherapy:

1. Vaccines;
2. Therapeutical and prophylactic antisera or immunoglobulins;
3. Immunomodulators (γ-IFN);

Complications of passive immunization and immunotherapy by antisera and Ig:

- Hypersensitivity (anaphylactic shock, serum sickness).
- Rick of HIV transmission (low).
- Rick of prions transmission.

Lecture 13. CLINICAL IMMUNOLOGY. TOLERANCE. TRANSPLACENTAL AND ANTITUMORAL IMMUNITY. AUTOIMMUNE DISEASES. IMMUNODEFICIENCIES

TOLERANCE

Tolerance is absence of the specific immune response in a presence of available specific Ag (*unresponsiveness*).

Ag is called **tolerogen.**

Types of tolerance:

– **Natural** (tolerance to own self Ag) → acquired during the fetal life.

– **Induced** → acquired during a life.

Development of tolerance:

– Contact of fetus with Ag during the prenatal life.

– High doses of circulating Ag.

– Molecular mimicry: resemblance between bacterial Ag and host Ag.

Mechanisms of tolerance:

1. Deletion of clones of B and T-cells by apoptosis (negative selection).
2. Inactivation of BCR and TCR.
3. Stopping the proliferation of the lymphocytes.

Application of tolerance in medicine:

- Depression of immunity during transplantation.
- Depression of autoimmune reactions.
- Treatment of allergy.

TRANSPLANTATION IMMUNITY

Transplantation (grafting) is process of moving cells, tissues, organs from donor to recipient. Object that is transplanted is called **graft.**

Types of graft:

• autograft – from one individual to same;

• syngeneic graft – from one identical twin to other;

• allograft – from one genetically different individual to other of the same species;

• xenograft – between two individuals of different species.

Graft rejection (GR) is a reaction of the recipient immune system against graft of donor (MHC antigens control the reaction of graft rejection).

Mechanism of GR:

1. CMI (CTL with CD8 recognize MHC I class of the transplantant cells ► specific cytotoxicity of graft).

2. AMI is additional mechanism.

Types of GR:

• hyper acute (within minutes or hours);

• acute (after 1 week);

• chronic (after several months).

Methods of stopping the GR:

1. Immunosuppression.
2. Detection of pre-existing Ab to graft.
3. Tissue typing (for HLA).
4. Induction of tolerance of recipient to graft.

GvHD (Graf-versus-Host Disease) or **GVH reaction** is immune response of new graft against bone marrow of host.

ANTITUMORAL IMMUNITY

Tumor is uncontrolled growth of tissue that affects homeostasis of organism and eventually kills this organism.

Malignant tumors are called **cancers.**

Tumor antigens:

1. Tumor-specific Ag (TSA) – unique for tumor only.
2. Tumor-associated Ag (TAA) – abnormal cell Ag.

Mechanisms of oncogenesis:

1. Mutation of host genes.
2. Activation of tumor suppressor genes of host.
3. Oncogenic viruses.

Immunity to tumors is provided by CTLs (kill viruses infected cells that have on their surface presented viral antigens!)**,** ADCC, NK-cells and macrophages. Tumors cam inhibit immune response of host.

IMMUNOPATHOLOGY

Immunopathology includes:

1. Hypersensitivity (see previous lecture).
2. Autoimmune diseases (autoimmunity).
3. Immunodeficiency.

AUTOIMMUNITY AND AUTOIMMUNE DISEASES

Autoimmunity is immune response against self Ag ("self" is recognized by the immune system as "non-self").

- basis – breakdown of natural tolerance;
- Ag is called **autoantigen;**
- mechanisms – AMI and CMI.

Autoimmune reaction develops during:

1. Damage of autoantigens;
2. Immune response on cross-reactive antigens;
3. Releasing of Ag during injury that in norm are isolated from immune system (extra barrier tissue Ag – localized in brain, testicles, anterior chamber of eye, crystalline lens).

4. Misbalance of regulation of immune system (atypical presentation, insufficient suppression, etc).

Classification of autoimmune diseases:

1.Systemic autoimmune diseases:

– rheumatoid arthritis;

– systemic Lupus Erythematosus (SLE);

– goodpasture syndrome.

2. Organ-specific autoimmune diseases:

– multiple sclerosis;

– myasthenia gravis;

– thyroiditis;

– diabetes;

– hepatitis;

– thrombocytopenia;

– hemolytic anemia.

IMMUNODEFICIENCIES (“defective immunity”)

Disorder of immune system when it can not to protect of the organism against pathogens and tumor cells.

Classification of immunodeficiency:

1. Primary (congenital) immunodeficiencies.

2. Secondary (acquired) immunodeficiencies.

3. Cellular, humoral or combined immunodeficiencies.

Congenital (primary) immunodeficiency

Genetic, hereditary disorders of immune system.

Types:

1. Congenital immunodeficiency of specific immunity (CMI, AMI):

– **immunodeficiency of B cells** (X-linked or Bruton’s agammaglobulinemia → absence of Ig and B-cells, hypogammaglobulinemia, Ig G deficiency).

– **immunodeficiency of T cells** (DiGeorge syndrome → absence of thymus and T cells);

– **severe Combined Immunodeficiency** (SCID).

2. Congenital immunodeficiency of innate immunity:

– **defects of phagocytes;**

– **defects of NK cells;**

– **defects of complement**.

Example: chronic granulomatous disease (CGD).

Acquired (secondary) immunodeficiency

More common than primary; acquired during life due different factors.

Factors leading to secondary immunodeficiency:

— Infections (influenza, HIV, rubella, hepatitis, TB, syphilis).
— Chronic noninfectious diseases.
— Malnutrition.
— Ionizing radiation.
— Tumors.
— Immunosuppressive drugs and hormones.
— Unhealthy mode of life.
— Distress, depression, trauma.
— Burns, long bleedings.

Examples of secondary immunodeficiencies

- Common hypogammaglobulinemia.
- AIDS (acquired immunodeficiency syndrome).
- Neutropenia.
- Chronic leukemia.
- Chronic Fatigue Immune Dysfunction Syndrome.
- Chronic pyoseptic infections of skin, RT, GIT, urogenital system.

CONTENTS

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