**MINISTRY OF HEALTH OF THE REPUBLIC OF KAZAKHSTAN**

**"SOUTH KAZAKHSTAN MEDICAL ACADEMY" JSC**

**Microbiology, Virology and Immunology Department**

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# LECTURE COMPLEX ON THE SUBJECT "MICROBIOLOGY AND IMMUNOLOGY"

## (General Microbiology)

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The lecture complex intended for 2nd year students studying in the specialty «General Medicine» in English. The content of the material on general medical microbiology in the proposed lecture complex includes eight lecture topics, which consistently address the issues of general microbiology and immunology. Lecture complex presents an overview of the history, modern state and perspectives of medical microbiology, describes the classification principles of microorganisms, features of the functional organization of the microbial cell, morphology and obiological features of microorganisms, their role in human pathology. It presents data on the physiology, genetics, and ecology of microorganisms, shows the strategy of antibiotic therapy and ways to prevent microbial resistance to antibiotics and present data on infections. A number of lectures were devoted to the issues of immunology, which revealed the structure of the immune system and the theory of immunity disclosed. There is also information about antigens and antibodies, serological reactions, and allergens. The theoretical material illustrated with tablesand figures. Each lecture accompanied by illustrative material and a list of references.

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## CONTEXT

## THE LIST OF ABBREVIATED WORDS

**UI** – Universal Indicator

**UV** – Ultraviolet

**DNA** – Deoxyribonucleic acid

**RNA** – ribonucleic acid

**r RNA** – Ribosomal RNA

**m RNA** – messenger RNA

**ATP** – adenosine triphosphate.

**GTP** – guanosine triphosphate

**MFA** – meclofenamic acid

**LIFA** – lanthanide immunofluorescence analysis

**RT-PCR** – Reverse transcription polymerase chain reaction

**CSF** – cerebrospinal fluid

**IFN** – Interferons

**MIC** – minimal inhibitory concentration

**MOC** – Minimum overwhelming concentration

**IPC** – Infection Prevention Control

**NCCLS** – National Committee for Clinical Laboratory Standards

**MBC** – The minimum bactericidal concentration

**LD50** – 50% lethal dose

**ID50** – 50% infectious dose

**LPS** – lipopolysaccharids

**CNS** – Central Nervous System

**IS** – The immune system

**NK** – Natural killer cells

**MBLs** – mannose-binding lectins

**Il** – interleukins

**AVPs** – synthesis of antiviral proteins

**TNF** – tumour necrosis factor

**Da** – daltons

**APCs** – antigen-presenting cells

**TH** – helper T

**TD** – T cell – dependent antigens

**TI** – T cell – independent antigens

**SAgs** – Superantigens

**MHC** – Major Histocompatibility Complex

**CRA** – Cross-reacting antigen

**Ig** – Immunoglobulin

**H** – Heavy chains

**L** – Light chains

**CIE** – Counter current immunoelectrophoresis

**HBsAg** – Hepatitis B surface antigen

**TPHA** – Treponema pallidium hemagglutination

**ASO** – Administrative Services Only

**CRP** – C-reactive protein

**ELISA** – enzyme linked immuno-sorbent assays

**SRS-A** – slow-reacting substance in anaphylaxis

**SLE** – Systemic lupus erythematosus

**TSH** – thyroid-stimulating hormone

## LECTURE № 1.

## TOPIC: GENERAL MICROBIOLOGY AND VIROLOGY MORPHOLOGY OF BACTERIA AND VIRUSES.

**1. Purpose:** to familiarize students with the subject and tasks of microbiology in their historical development, the history of microbiology, virology and immunology, as well as on the taxonomy, morphology and ultrastructure of microorganisms.

**2. Abstracts of the lecture.**

**The subject of microbiology** is a special world of living beings, invisible to the naked eye and having sizes ranging from 1-10 nm to 0.1-1 µm.

Microorganisms include several different groups of pathogens - ***bacteria, viruses, protozoa, fungi*** **(Figure 1.1).**

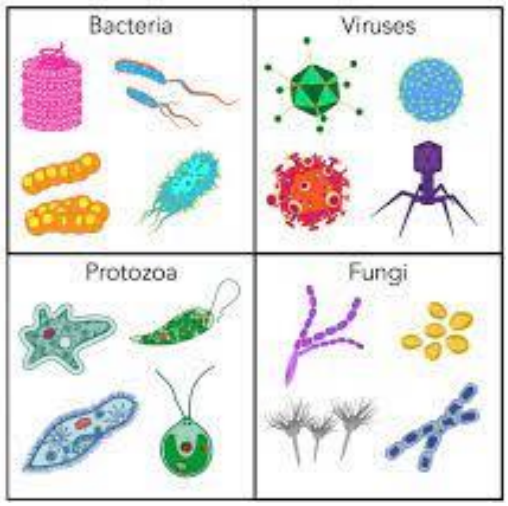


**Figure 1.1 Types of microorganism:** *bacteria, viruses, protozoa, fungi*

**Microbiology** – is the science that studies the life and increase of the smallest living beings - microorganisms - together with their complex relationships with the environment [1].

**Tasks of microbiology and immunology:**

• to give an idea of the classification and biological properties of pathogenic and conditionally pathogenic microorganisms**;**

• to familiarize with modern methods of microbiological diagnostics of common infectious and non-infectious diseases of microbial etiology and to give an idea of the structure of the immune system.

Given the huge role that microorganisms play in nature, the problems of microbiology are quite diverse. Microbiology constantly differentiated into various scientific sections and disciplines.

**Modern** microbiology includes ***general*** microbiology (examines the general principles of structural organization and general functions of microorganisms), ***private*** microbiology (conducts a detailed study of certain microbial agents and groups); ***industrial*** microbiology, which is the main part of modern ***biotechnology***; ***agricultural*** microbiology; ***space*** microbiology; ***sanitary*** microbiology; ***veterinary*** microbiology; and ***medical*** microbiology **(Figure 1.2).**

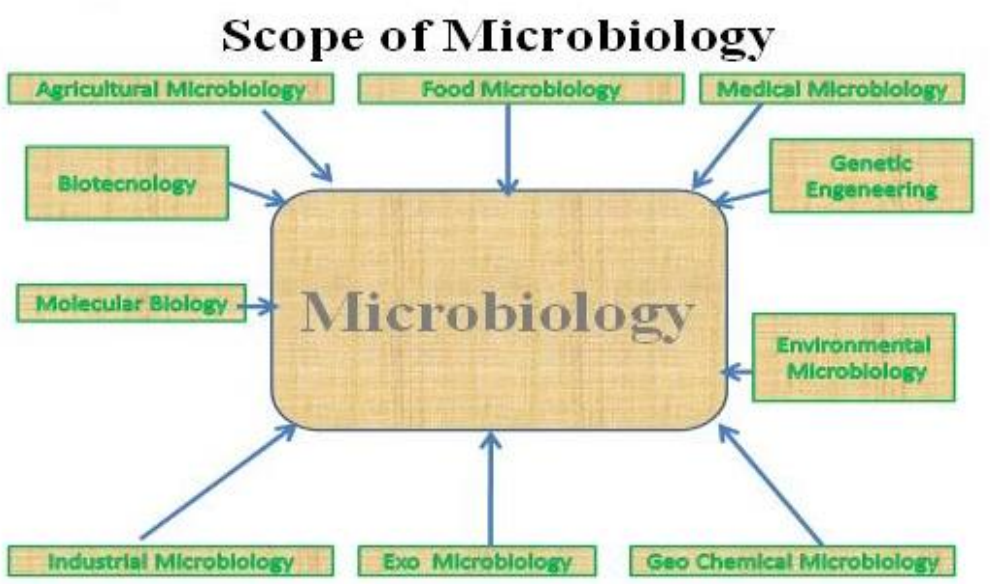


**Figure 1.2 Sphere of microbiology**

The ***subject of medical microbiology*** covers pathogenic microorganisms that cause diseases in humans, and those non-pathogenic microorganisms – inhabitants of living beings or the external environment - that can affect human health [1].

**Microbiology** (*gr. micros – small, lat. bios – life*) is a branch of biological sciences that studies morphology, systematics, physiology, genetics, ecology of microorganisms and relationships with other organisms inhabiting our planet.

Microorganisms are the oldest form of life on Earth, they appeared long before the appearance of plants and animals - about 3-4 billion years ago. Currently, microorganisms represent a number of the most numerous and diverse part of the organisms inhabiting the Earth's biosphere. This served as the basis for the division of all microorganisms into four great kingdoms: bacteria, fungi, protozoa and viruses. Each of them is the object of study of separate sections of microbiology, independent disciplines of bacteriology, virology, mycology, protozoology and allergology. During the history of microbiology, original research methods had been develop, adopted from other disciplines – biophysics, biochemistry, genetics, cytology, etc. [2].

**The main purpose of medical microbiology:**

1. Laboratory diagnostics of diseases caused by microorganisms using universal microbiological methods; identification of pathogenic microbial agents in living organisms and the external environment.

2. Sanitary control of microbial contamination of water, air, soil, home, food, medicines, etc.

3. Development of biological preparations for medicine (antibiotics, vaccines, immune serums, polyclonal and monoclonal antibodies, cytokines and others), which are used for the prevention and treatment of bacterial, viral, fungal and protozoal diseases; autoimmune and inflammatory diseases [1].

## HISTORICAL PERIODS IN THE FORMATION AND DEVELOPMENT OF MICROBIOLOGY

**I. The beginning period.** Human encountered pathogenic microorganisms at the dawn of his development. Long-term monitoring of infectious diseases made it

possible to make important discoveries, and sources of that time pointed to the role of hygienic rules for their prevention. The discovery of the world of microorganisms covers the period of the second half of the XVIII century – the middle of the XIX century. This is due to A. Leeuwenhoek's creation of a simple microscope and the discovery of microscopic creatures invisible to the human eye.

**Figure 1.3 Anthony van Leeuwenhoek -** discoverer of microorganisms

**II. The Pasteur period** of the «*golden age*» related with the name of Louis Pasteur described by the formation and development of microbiology and immunology as independent combined natural sciences with their own objects and original methods of their study [2, 3].

***The Discoveries of Pasteur:***

- Established that the processes of fermentation are microbiological nature, and each type of fermentation is cause by its specific pathogen.
- Exploring the disease of beer and wine, he discovered that these defects are due to the development of extraneous microorganisms. He proposed a method of suppression of extraneous microflora – pasteurization.
- Explained that infectious diseases have microbial nature and occur as a result of from the ingestion of pathogens.
- Louis Pasteur proposed a method of combating infectious diseases using vaccinations, which used cultures of microorganisms with weakened pathogenic action (vaccines)
- Proved that some microorganisms can exist without access oxygen that discovered the phenomenon of anaerobiosis. In studying butyric bacteria, he

showed that air is harmful for them. These results provoked a storm of protest, as it was recognize that no molecular oxygen is essential for life [5].

**Figure 1.4 Louis Pasteur –** founder of the science of microbiology

**III. The third period** covering the first half of the XX century, characterized by the further development of microbiology and immunology and the establishment of virology as well as means of dealing with the causes of infectious diseases, improvement of diagnostic methods.

**Figure 1.5 Dmitry Ivanovsky –** founder of the science of virology

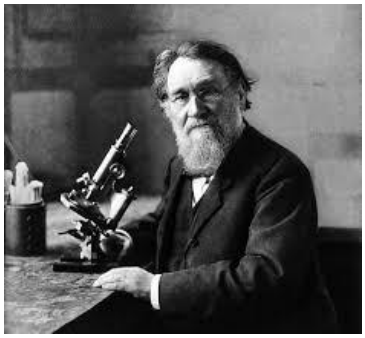

**Figure 1.6 Ilya Ilyich Mechnikov** – developer of the cellular theory of immunity

**IV. The modern period,** which began in the middle of the twentieth century with the scientific and technological revolution in the natural sciences. The subject of study medical microbiology is pathogenic and conditionally pathogenic microorganisms for humans, as well as the development of methods for microbiological diagnosis, specific prevention and treatment of infectious diseases caused by them. At the same time, medical microbiology and immunology has been form, which investigate specific mechanisms of protecting humans and animals from pathogens and other problems [2, 3].

## CLASSIFICATION OF MICROORGANISM

Classification of living beings is one of the most difficult sections of biological science. In it, as in a focus, concentrate all our knowledge about organisms. The deeper and more complete our information about the organisms, the more precisely we classify them. With the progress of biological science improved and the classification of living things.

As the study of the biology of bacteria, researchers have begun to apply for the classification, morphological in addition to many other symptoms: physiological, biochemical, cytological, serological, immunological, etc. In modern classifications, the authors use any sign, but he stood out and gave the ability to recognize the studied organism [4].

**Principles of Microbial Classification**

The science that studies classification is calls taxonomy. It includes three interrelated areas:

**1. Classification -** distribution of microorganisms into groups with similar characteristics

**2. Nomenclature -** name of microorganisms in accorance with international requirements

**3. Identification -** comparison of unknown microorganisms with already classified.

The following taxonomic categories recommended by the decision of the International Code of Bacteria: *class, department, order, family, genus, species.*

The name of the species corresponds to a binary nomenclature, i.e., consists of two words. ***For example***, the pathogen of typhoid fever was write as Salmonella typhi.

**General properties of microorganisms** are:

- small size (dimensions of organisms are measured in μm 1 μm = $1^{-6}$ m);
- high rate of metabolic processes. This is due to the wide ratio of surface metabolism to cell volume. For microorganisms, the whole surface of the cell is a

surface exchange. As the cells of the bacteria very small, they are growing and developing faster all of the microorganisms, followed by yeasts and fungi. In turn, the rate of metabolism of the microorganisms in the tens and hundreds of thousands of times higher than animals.

- widespread in nature. The small size of microorganisms are important to the environment. Microorganisms can be disseminated by air currents and are everywhere;
- the plasticity of the exchange – high adaptability (adaptation to new conditions of existence). The much greater flexibility of metabolic processes in microorganisms compared to plants and animals is due to their capability to synthesize induced enzymes, i.e. enzymes that are produced in the cell only in the presence of appropriate substances in the environment.;
- a high degree of variability. A higher degree of variability of microorganisms compared with microorganisms is related to the fact that most microorganisms are single-celled organisms. On a separate cage to work is easier than on the body, consisting of many cells. A high degree of variability, rapid growth and development, high rate of metabolism, the formation of numerous posterity – all of these properties of microorganisms make them extremely convenient subjects for genetic analysis since experiments can be performed in a short time on a huge number of individuals. [6].

However, these organisms differ in significant ways from each other in many ways and especially in terms of organization of genomes, the availability and composition synthesizing protein systems and cell walls.

In accordance with these features, all known living beings are divided into 4 kingdoms: eukaryotes, eubacteria, archaebacteria, viruses and plasmids. They occupy the lowest rung of evolution, but play an important and varied role in the general economy of nature, in circulation substances, the pathology of man, animals and plants.

*Distinctive features of listed kingdoms of life the next:*

- The kingdom of viruses and plasmids includes organisms whose genome consists of either DNA or RNA; they do not have their own system of protein biosynthesis and energy mobilization therefore they are absolute intracellular parasites.

- Prokaryotes (Eubacteria and archaebacteria) are organisms that have not yet decorated the nucleus, but only its predecessor - the nucleoid. It is one or more chromosomes that consist of DNA and are freely suspended in the cytoplasm, which is not separated from any membrane. Prokaryotes do not have a differentiated mitosis equipment they do not have a nucleolus. In addition, they have S70 ribosomes, most of which have a cell wall containing peptidoglycan, which is absent in eukaryotes. Prokaryotes do not have mitochondria and chloroplasts. Among them there are aerobic and anaerobic organisms [5].

- Archaebacteria – live in habitats with extreme conditions. From eubacteria, they are distinguished by differences in the structure of the cell wall (no peptidoglycan layer); ribosomes, ribosomal enzymes, and RNA transport [3].

- Eukaryotes are organisms whose cells contain a nucleus. Eukaryotes include all higher plants and animals, as well as unicellular and multicellular algae, fungi, protozoa. Nuclear DNA in eukaryotes is concluded in chromosomes, usually not having a ring-shaped shape, with histones. Eukaryotes have membrane–limited cellular organelles - mitochondria, chloroplasts, etc. [7].

**Table 1-1 Classification of microorganisms**

| **Non-cellular forms** | **Cellular forms** | | |
|---|---|---|---|
| | Prokaryotes | Eukaryotes | |
| **Viruses –** can be exist in two forms: extracellular (virion) and intracellular (virus).<br>The size: from 15-18 to300-400 nanometers.<br><br>1nm=$10^{-3}$ µm | **Bacteria –** are unicellular microorganisms of plant origin, devoid of chlorophyll and do not have nucleus.<br>The size: from 0.3 to 5-10 micrometers.<br><br>1µm =$10^{-3}$ mm | **Protozoa –** are unicellular animals organisms.<br>The size: from 2 to 50 micrometers. | **Fungi** – are unicellular and multicellular organisms of plant origin. Devoid of chlorophyll, but with the same features as animal cells<br>The size: from 0.2 to 100 micrometers. |

Specific features of the microorganisms identified and the range of characteristics and properties that are used for their taxonomy and classification.

- **Morphological characteristics** – size, shape, nature of the relationship
- **Tinctorial properties** – the ability to be painted different colors. A particularly important feature has to do with the type of gram staining, which turn on the structure and chemical structure of the bacterial cell walls. On this basis, all bacteria are separated into gram-positive and gram-negative. Morphological properties and the relationship with the type of gram staining to determine belonging to the main taxa – genus, family, etc.
- **Cultural properties** – characteristics of bacteria growth in liquid (film formation, sediment, turbidity) and dense (shape, size, consistency, edge, surface, transparent colonies, pigment formation, and other properties) nutrient media.
- **The mobility of bacteria.** There are moving and stationary bacteria. Motile bacteria are separated into creeping or sliding they are set in motion by wave-like contractions of cells; and floating bacteria, in which active mobility is related with the presence of flagella.
- **Sporulation** – the shape and location of spores in the cage.
- **Physiological properties** – carbon ways (autotroph, heterotrophs), azotovit (aminoacetate, amino heterocycles) power; type of respiration: aerobic, facultative anaerobes, strict anaerobes, microaerophile.
- **Biochemical properties** – the ability to ferment various carbohydrates, proteolytic activity, formation of indole, hydrogen sulfide, presence of urease and other enzymes, etc.
- **Sensitivity to specific phages.**
- **The antigenic properties.** Depend on the chemical structure of the cell wall and flagella of bacteria.
- **The chemical structure of the cell walls** (the content and composition of major sugars and amino acids).

- **Lipid and fatty acid composition.** The study of the composition of fatty acids is performed by gas chromatography which has high separation capacity and sensitivity.

- **Protein spectra.** Using various methods of fractionation, and mainly two-dimensional polyacrylamide gel electrophoresis, separate complex mixtures ribosomal, membrane or intracellular proteins and get electroforegrams, or protein spectra corresponding to fractions of this bacteria. [5].

**Principles of systematization of bacteria in the determinant burgee**

The Berge determinant systematizes all known bacteria in accordance with the principles of bacterial identification used in practical bacteriology, based on differences in the structure of the cell wall and Gram staining. The determinant identifies four main categories of bacteria:

- ***Gracillicutes*** - species with a thin cell wall, stained gram-negative;
- ***Firmicutes*** - bacteria with a thick cell wall that stain gram-positive;
- ***Tenericutes*** - bacteria lacking the cell wall;
- ***Mendosicutes*** - archaebacteria (defective cell wall).

## GENERAL VIROLOGY

The rapid pace of development of Virology in the second half of the twentieth century permitted us to obtain important information about the structure and chemical structure of various viruses, including their genome, as well as the nature of relationship with host cells.

The obtained materials indicate that viruses exist in two qualitatively different forms: extracellular – and intracellular virion – virus. The virion of the simplest virus is a nucleoprotein, which includes the viral genome protected by protein shell – the capsid. At the same time, intracellular virus is self-replicating form, is not capable of binary fission. [2].

Morphology of microorganisms, studying their appearance, form and structure features, the ability to move, sporulation, methods of reproduction.

**VIRUSES**

Viruses are very simple microbes, consisting of nucleic acid, a few proteins, and (in some) a lipid envelope. These microbes are completely dependent on the cells they infect for their survival and replication. Medically important viruses are subdivided into 20 families defined by the structural properties of the members. The most important feature is the nucleic acid. Viruses contain either DNA or RNA but not both. The families of DNA viruses and RNA viruses are further subdivided into viruses with either single-stranded or double-stranded nucleic acids. Lastly, these viral families are further subdivided into viruses with an outer envelope, or naked non-enveloped viruses. Well, the viruses are further subdivided by their shape (spherical or rod shape) and size (big or small) [8].

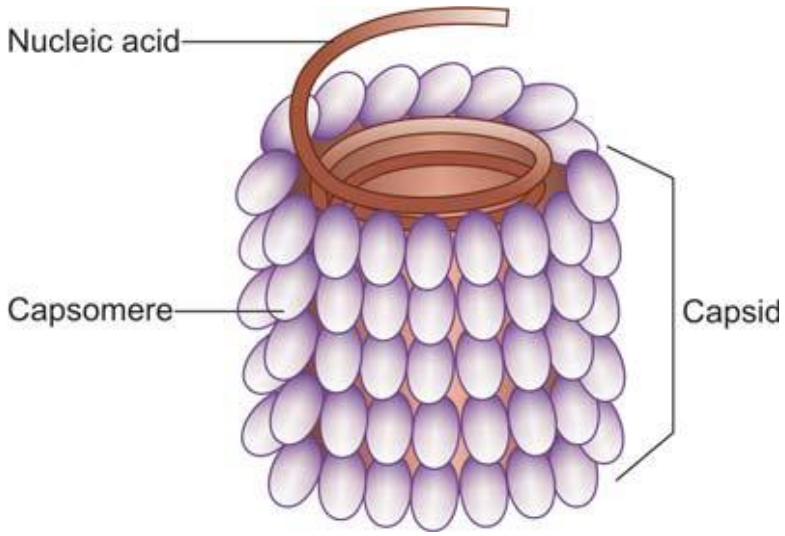


**Figure 1.7 The structure of the viral particles:** Nucleic acid, capsomere and capsid

The structure of viruses (Figure 1.7) can be subdivided into 4 types, which vary in the nature of the packaging of morphological subunits:

- viruses with spiral symmetry;
- viruses with isometric curved and cubic symmetry;
- viruses with binary symmetry, for example, phages: their head has cubic symmetry, and the tail is helical;
- organized into more complex viruses have a second shell.

Currently, for the classification of viruses used the subsequent criteria.

- Nucleic acid: type, number of strands, percentage, molecular weight, guanine and cytosine content.
- Morphology: the type of laws of symmetry or pseudo-symmetry, the number of capsomers for viruses with cubic symmetry, the presence of a lipoprotein outer shell, the shape and size of virions.
- Biophysical properties: permanent deposition, buoyant density.
- Proteins: the number of structural proteins, their localization, amino acid composition.
- Lipid composition.
- Reproduction in tissue cultures, particularlyr eplication.
- The circle of target hosts, the pathogenesis of the infectious process; oncogenic properties.

Resistance to physical and chemical factors (gamma radiation, thermal activation at 37°C and 50°C, the action of liposolvent and individual cations).

- Antigenic properties.

The name of all virus genera ends with the word "virus", the suffix "*idae*" is used for the names of families and subfamilies are "*inae*" [5].

***Chemical structure of virions***

Simple virions contain one type of nucleic acid – RNA or DNA and proteins. In complex virions consisting of an outer shell containing lipids and polysaccharides, the former is produced from the host cell, the glycoprotein is encoded by the virus genome.

**BACTERIOPHAGES (BACTERIA VIRUSES)**

Bacteriophages are viruses that infect bacteria. The F.D. Herelle (French scientist) was the first who discovered bacteriophages.

The phages have very complicated structure and are called **complex viruses**. They have the shape of a tadpole or a sperm.

They consist **of *head, neck, tail*** and ***basal plate*** from each angle of which six teeth and filaments going out. Due to these, phage is adsorbed on the bacteria cell.

The nucleic acid of DNA or RNA is contained in the hexagonal head. The head is surrounded by a protein membrane or capsid. The neck is free of this membrane. The tail consists of a hollow nucleus surrounded by a protein membrane and has cylindrical symmetry, and a free strand of nucleic acid is suspended in this cylinder. The enzyme lysozyme is contained in the basal lamina. Due to this, the lysozyme phage destroys part of the bacterial cell wall and penetrates through the bacterial cell wall.

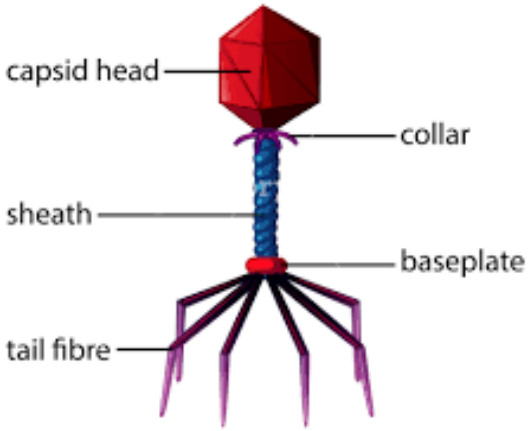


**Figure 1.8 The structure of the bacteriophages**

1. The interaction of phage and bacteria is manifested in the following phases occurring in succession:
    - Adsorption
    - Penetration into the cell
    - Reproduction: biosynthesis, maturation
    - Release

2. **Adsorption.** After a chance collision between phage particles and bacteria, attachment, or adsorption, occurs. During this process, the attachment site on the virus attaches to a complimentary receptor site on the bacterial cell. This attachment is a chemical interaction in which weak bonds are formed between the

attachment and receptor sites. The complementary receptor sites are on the bacterial cell wall.

3. **Penetration.** After the attachment the bacteriophage injects its nucleic acid into the bacterium. To do this, bacteriophage's tail releases an enzyme, *phage lysozyme*, which breaks down a portion of the bacterial cell wall. In the process of penetration, the tail shell of the phage contracts, and the tail nucleus passes through the cell wall.

4. When the tip of the core reaches the plasma membrane, the DNA from the bacteriophage's head passes through the tail core and through the plasma membrane and enters the bacterial cell.

5. **Biosynthesis.** Is the process when the nucleic acid is integrated in the bacterial genetic material and the synthesis of substrates necessary for the phage takes place. Initially, the phage uses the host cell's nucleotides and several of its enzymes to synthesize many copies of phage DNA. Soon after, the biosynthesis of the viral proteins begins.

6. **Maturation and Release**. In the next sequence of events, maturation occurs. In this process, bacteriophage DNA and capsids are assembled into complete virions. The phage heads and tails are separately assembled from protein subunits and the head is filled with phage DNA and attached to the tail. After self-collection the phages are released from the bacterial cell and affect that cell. These viruses are termed **virulent** phages. In contrast to virulent bacteriophage some viruses do not cause lysis and death of host cell when they multiply. These ones called **temperate phages** penetrate into the bacterial cell then integrate in the genetic material of the bacteria (bacterial chromosome). The integrated into nucleic acid phage is known as a prophage. The prophage behaves like a segment of the host chromosome and replicates with it. This phenomenon is called ***lysogeny***, and a bacterium carrying a prophage in its genome is called a ***lysogenic bacterium.***

The lysogenic bacterium acquires new properties. This phenomenon is known as lysogenic conversion or phage conversion. The result of lysogeny is that the

host cell can exhibit new properties. For example, the bacterium Corynebacterium diphtheriae, the causative agent of diphtheria, is a pathogen whose disease–producing properties are associated with the synthesis of the toxin. An organism can produce a toxin only when it is a carrier of a moderate phage, because the phage carries the gene encoding the toxin. Streptococci carrying a moderate phage are capable of producing a toxin that causes scarlet fever. The toxin produced by Clostridium botulinum, which causes botulism, is encoded by the prophage gene.

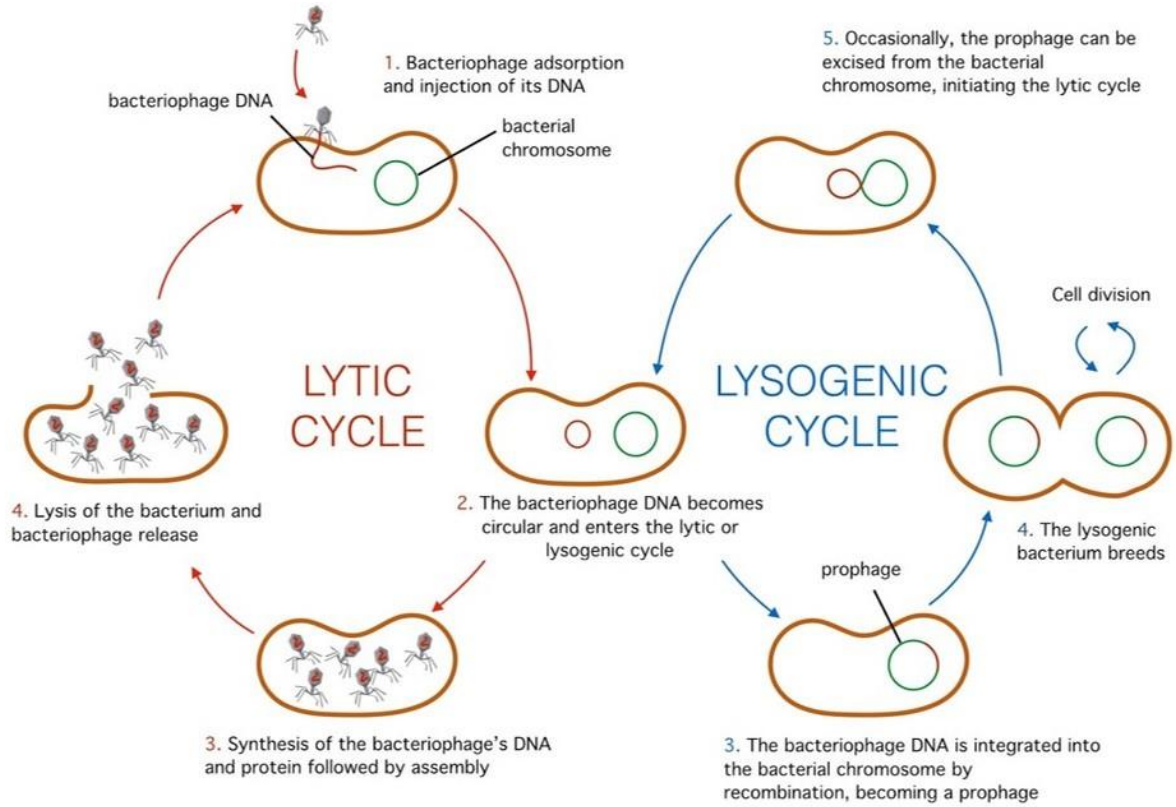


**Figure 1.9 life cycle of bacteriophage**

Phages are used in the **prevention** and **treatment** of infectious diseases, and are also used in the **diagnosis** of certain infectious diseases [2, 3].

**BACTERIA**

Bacteria are a bit more complex, with both RNA and DNA, metabolic machinery for self-replication, and a complex cell wall structure. Bacteria are prokaryotic organisms; that is, simple unicellular organisms with no nuclear membrane, mitochondria, Golgi bodies, or endoplasmic reticulum and they

reproduce by asexual division. The key feature that is used to separate most bacteria is their staining property, with the Gram stain and acid-fast stain the most important. Most bacteria are either gram-positive (retain the blue dye) or gram-negative (lose the blue stain and stain with the red dye). These bacteria are then subdivided by their shape (either spherical [cocci] or rod-shaped), whether they grow aerobically or anaerobically (many bacteria grow in both atmospheres and are called facultative anaerobes), and whether they form resilient spores or not (only gram-positive rods are spore-formers). The other important bacterial stain is the acid-fast stain that is retained only by a few bacteria that have a characteristic lipid-rich cell wall. This group is further subdivided by how difficult it is to remove the acid-fast stain (the stain is named because an acid solution removes the stain from most other bacteria). Finally, there are groups of organisms that are not stained using these procedures, so they are separated by other characteristics, such as shape (spiral-shaped bacteria) or their need to grow inside a host cell (for example, a leukocyte) or cell cultures in the laboratory [8].

**Shape of bacteria**

**Basic shapes of bacteria**: spherical, rod-shaped and spirochetes.

***Spherical bacteria*** - cocci are subdivided into six groups according to plan of cell division, cell arrangement; and biological properties.

1. **Micrococci** (micrococcus) - divide in one plane. The cells are arranged singly or irregularly. They are saprophytes and live in water and in air.

2. **Diplococci** (GK-diplos-double) - are divided in one plane and remain attached in pairs. These include: meningococcus, the causative agent of epidemic spinal meningitis, and gonococci, the causative agents of gonorrhea and blennorrhea. These cocci resemble coffee beans. Another diplococcus is pneumococcus– the causative agent of pneumonia. This diplococcus is similar to a lancet.

3. **Streptococci** (GK–Streptos-chain) divide in one plane and are arranged in chains of different length. They are probably responsible for a number of illnesses

and cause a greater variety of diseases than any other group of bacteria. Scarlet fever, pharyngitis (sore throat) and others are among the diseases caused by streptococci.

4. **Tetracocci** (GK–tetra–four) divide in two planes and form groups of fours. They are saprophytes.

5. **Sarcinae** (GK-Cartio-to tie) - divide in three planes and resemble packets of 8, 16 or more cells. They are frequently found in the air. These are conditionally pathogenic organisms.

6. **Staphylococci** (GK–Staphyle–cluster of grapes) - divide in several planes resulting in irregular bunch of cells, sometimes resembling clusters of grapes. The most important staphylococcal species is S. aureus, named for its yellow pigmented colonies (aureus means golden), causative agents of diseases in man and animals. S. aureus produced many toxins that contribute to the bacterium's pathogenicity by increasing its ability to invade the body or damage tissue.

**Rods -** rod-shaped or cylindrical forms are subdivided into **bacteria** (include those micro-organisms which as a rule do not produce spores – e.g. organisms responsible for enteric fever-paratyphoids, dysentery, diphtheria, etc.) **bacilli and clostridia (spindle form)** include organisms the majority of which produce spores (e.g. bacilli responsible for anthrax, clostridia - tetanus, and etc.). Bacillus (B. anthracis) is rod shape spore forming aerobic microorganism. Clostridium (C. tetani, C. botulinum) are rod shape, spore forming, anaerobic microorganisms. Rods exhibit **differences** in the form of ends, in shape and size as well as in arrangement. According to the shape of the end, they can be rounded, pointed, thickened, truncated, etc. By size they may be small (0.5-1.5μm) middle (2-5μm) and large (6-10μm). By their arrangement they may be irregular arranged, in pairs, in chains.

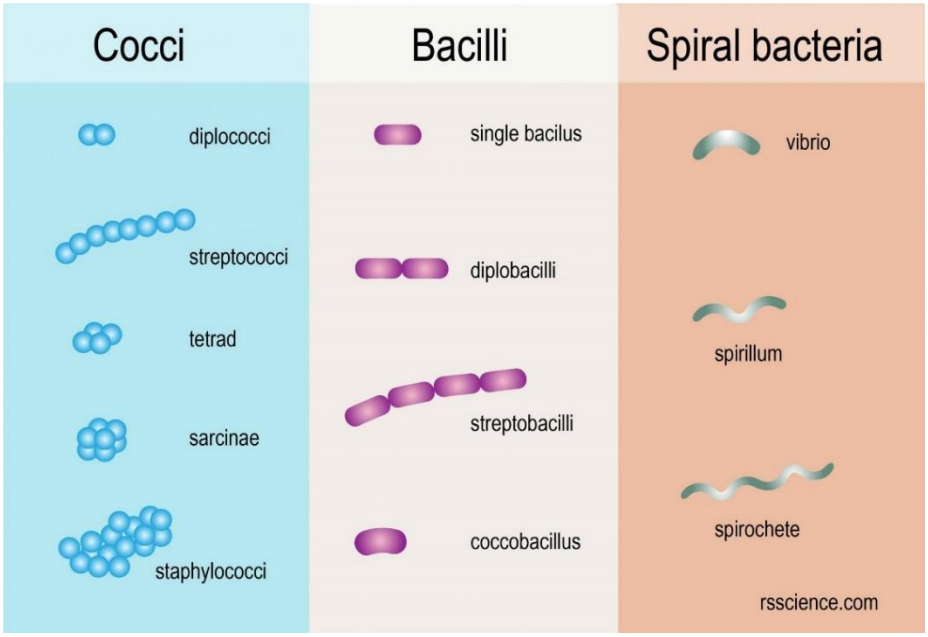


**Figure 1.10 Basic shapes of bacteria**

**Spiral shaped bacteria** – *Vibrions, Spirilla and Spirochetes* belong to this group of bacteria. Spirillae are coiled forms of bacteria that exhibit bends with one or more turns. There is only one known pathogenic species (Spirillum minus), which causes a disease in humans transmitted through the bites of rats and other rodents (Sodoku).

**Size of bacteria:** Most of bacteria are so small that their size is measured in terms of micron. 1 micron (µ) or micrometre (µm) = One-thousandth of millimetre. 1 millimicron (mµ) or nanometre (nm) = One-thousandth of micron. 1 Angstrom units (Å) = One-tenth of nanometre. Generally, cocci are about 1 µ in diameter and bacilli are 2 to 10 µ in length and 0.2 to 0.5 µ in width. The limit of resolution with unaided eye is about 200 µ. Obviously, bacteria can only be visualized when magnified (Figure 1.11).

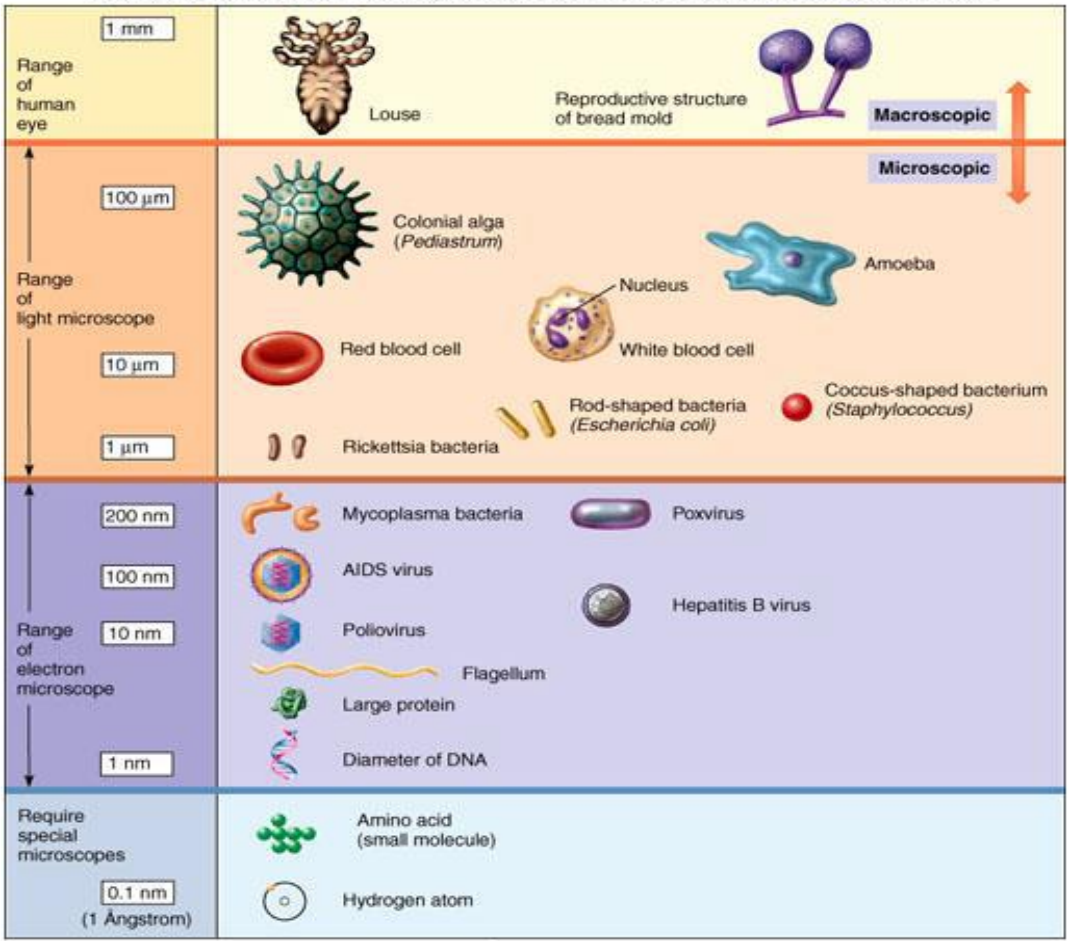


**Figure 1.11 Size of microorganisms**

## MORPHOLOGY, ULTRASTRUCTURE AND FUNCTIONS OF BACTERIA

Bacteria differ essentially from plant and animal cells in structure. They consist of the main and supplementary (additional) structures: The main structures are:

- cell membrane
- cytoplasm
- nucleoid

The supplementary (additional) structures are:

- flagella
- pili (fimbriae)
- Spores

**The cell membrane consists of three components**:

**1.** the external layer – glycocalyx which may form a capsule

**2.** the middle layer – cell wall

**3.** the internal layer – cytoplasmic membrane

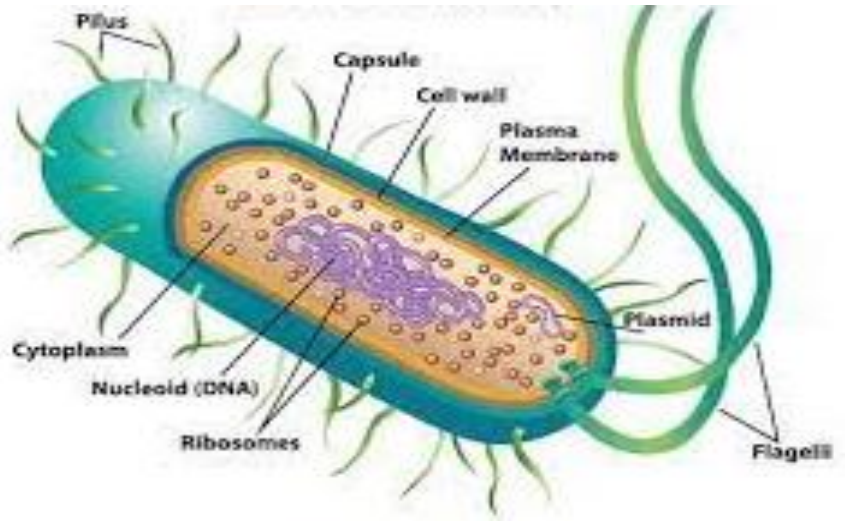


**Figure 1.12 Bacterial cell structure**

**CAPSULE (the external layer) -** glycocalyx is a viscous (sticky) gelatinous polymer that is located outside the cell wall and consists of a polysaccharide, a polypeptide, or both. Its chemical composition varies greatly depending on the species. In some bacteria, this outer layer can take the form of a ***capsule***. Capsule usually forms in human or animal organisms (B. anthracis, S. pneumonia, etc.), but some bacteria can form capsule in nutrient media too (eg. K. pneumonia, K. ozenae, K. rhinoscleromatis), which called **constantly** capsule forming bacteria (capsular). The capsule is composed of: **polysaccharides** (Streptococcus pneumonia, Clostridium perfringens); **polypeptides** (Bacillus anthracis, Yersinia pestis).

**Capsule forming bacteria are:** Streptococcus pneumonia, Clostridium perfringens, Klebsiella pneumonia, Klebsiella ozenae, Klebsiella rhinoscleromatis, Neisseria meningitis, Bacillus anthracis , Yersinia pestis.

**The functions of capsule are:**

1. **Protective:** protection of microorganism from biological, physical, chemical agents (phagocytosis, antibodies, antibacterial drugs, drying.)
2. **Virulence**: it is determinant of virulence of many bacteria, since it limits the ability of phagocytes to engulf the bacteria. Loss of the capsule may render the bacterium avirulent.
3. **Antigenicity:** capsular polysaccharides and polypeptides ensure the antigenicity.
4. **Adhesive:** The capsule may play a role in the adherence of bacteria to human tissues, which is an important initial step in causing infection.

Capsule cannot be stained with ordinary stains like gram staining. It can be visualized by **Ionne, Burry-Hins** staining methods and it may also be visualized by reaction with specific antibody (capsular material is antigenic and may be demonstrated by serological methods) which causes a characteristic swelling of the capsule. It is known as **Quellung reaction.** This phenomenon is seen in and allows rapid identification of capsular serotypes of Streptococcus pneumoniae, Neisseria meningitidis and etc. [2,8,9,10].

**The cell wall** is a tough and rigid structure surrounding the bacterium. It has a thickness of 10-25 nm and weighs about 20-25% of the dry weight of the cell.

**The cell wall has following functions:**

- It provides protection to the cell against osmotic lysis;
- It confers rigidity upon bacteria due to presence of peptidoglycan layer in the cell wall;
- The cell wall can protect a cell from toxic substances and is the site of action of several antibiotics;
- Virulence factors: Bacterial cell wall contains certain virulence factors (e.g. endotoxin), which contribute to their pathogenicity;
- Immunity: Antibody raised against specific cell wall antigens (e.g. antibody to LPS) may provide immunity against some bacterial infection.

**Gram-positive Cell Wall**. Cell wall of gram-positive bacteria is simpler than that of gram-negative bacteria. ***Peptidoglycan.*** In gram-positive bacteria, the peptidoglycan layer is much thicker (50–100 layers thick, 16–80 nm) than gram-negative cell wall. ***Teichoic Acid.*** Gram-positive cell wall contains significant amount of teichoic acid which is absent in gram-negative bacteria.

**Gram-negative Cell Wall**. Gram-negative cell wall is thinner and more complex than the gram-positive cell wall, comprises of the following components. ***Peptidoglycan Layer.*** It is very thin (1–2 layer, 2 nm thick), composed of a mucopeptide chain similar to that of gram-positive cell wall, and consists of alternate NAM and NAG molecules. ***Outer Membrane***. It is a phospholipid layer that lies outside the thin peptidoglycan layer; firmly attached to the latter by a membrane protein called Brown lipoprotein. ***Lipopolysaccharide (LPS).*** This layer is unique to gram-negative bacteria, which is absent in gram-positives. ***Periplasmic Space.*** It is the space between the inner cell membrane and outer membrane. It encompasses the peptidoglycan layer [10].

**The plasma (cytoplasmic) membrane** – or inner membrane - is a thin structure lying inside the cell wall and surrounding the cytoplasm of the cell. Electron microscopy shows the presence of three layers: two-layer phospholipids and a protein layer. It acts as a semipermeable membrane that controls the inflow and outflow of metabolites into and out of the protoplasm. It provides passive diffusion in and out of water and other low molecular weight substances, but actively affects the selective transport of certain nutrients into the cell and the removal of waste products from it.

**Mesosome** is the invagination of the cytoplasmic membrane into the cytoplasm. The mesosome plays an important role during cell division. They are the main centers of respiratory enzymes in bacteria and are similar to the mitochondria of eukaryotes and are more noticeable in gram-positive bacteria. They participate in sporulation.

**Cytoplasm** is about 80% water and contains primarily proteins (enzymes), carbohydrates, lipids; inorganic ions are present in cytoplasm. The cytoplasm is thick, watery, translucent and elastic. The main structures in the cytoplasm are DNA, particles called ribosomes, and reserve deposits called inclusions, plasmids.

**Ribosomes -** all eukaryotic and prokaryotic cells contain ribosomes (60% RNA and 40% proteins), which function as the sites of protein synthesis. But prokaryotic cells differ from eukaryotic ribosomes in size and chemical composition. Bacterial ribosomes have a size of 70S (with 50S and 30S subunits), whereas eukaryotic ribosomes have a size of 80S (with 60S and 40S subunits). The letters refer to the Svedberg units, which indicate the relative deposition rate during ultra-high-speed centrifugation. The deposition rate depends on the size, weight and shape of the particle. Some antibiotics act by inhibiting protein synthesis on ribosomes. Each cell contains thousands of ribosomes strung together on messenger RNA (mRNA) strands, forming polysomes, and it is at this point that the mRNA code is translated into peptide sequences. Some antibiotics, such as neomycin, streptomycin, act by inhibiting protein synthesis on ribosomes. Due to differences in prokaryotic and eukaryotic ribosomes, the microbial cell can be killed by an antibiotic, while the eukaryotic host cell remains unaffected.

**Inclusions** – the cytoplasm contains several different types of granules (inclusions) that serve as storage sites for nutrients. Some inclusions are common to a wide range of bacteria (for example, polysaccharide granules-glycogen, lipid inclusions, etc.), while others are limited to a small number of species and therefore serve as a basis for identification. For example, volutin granules (metachromatic granules, Babesch–Ernst granules) – these large inclusions, which got their name due to the fact that they are sometimes colored red by certain blue dyes, such as methylene blue, are collectively known as *volutin granules*. **Volutin granules** are a reserve of inorganic phosphate (polyphosphate), which can be used in the synthesis of ATP. It is usually formed by cells that grow in a phosphate-rich environment.

Bacterial **nuclear area or nucleoid (**bacterial chromosome) contains a single long ring molecule of double-stranded DNA and a smaller amount of RNA. This is the genetic information of the cell, which carries all the information necessary for the structures and functions of the cell. Unlike the chromosomes of eukaryotic cells, bacterial chromosomes do not include histones and are not surrounded by a nuclear envelope (membrane), do not include a nucleolus, there is no mitotic apparatus.

**Plasmids** are extra-chromosomal double-stranded ring DNA molecules that are able to replicate independently of the bacterial chromosome. Although plasmids are usually extrachromosomal, they can be integrated into the bacterial chromosome. Plasmids can be obtained or lost without harming the cell. However, under certain conditions, plasmids are an advantage for cells. Plasmids can carry genes for activities such as antibiotic resistance (R-plasmids), tolerance to toxic metals, toxin production (toxoplasmids) and enzyme synthesis. Plasmids can be transmitted from one bacterium to another. In fact, plasmid DNA is used to manipulate genes in biotechnology.

**Flagella –** are long, whip–like appendages that move bacteria to nutrients and other attractants, a process called chemotaxis. The long thread that acts as a propeller consists of many subunits of a single protein - flagellin, which belongs to the same chemical group as myosin, a contractile muscle protein consisting of several intertwined chains. The energy for movement, the proton, the driving force is provided by adenosine triphosphate (ATP), obtained as a result of the passage of ions through the membrane.

**The functions of flagella:** gives motility to bacteria**;** gives antigenicity to bacteria (flagellar “H” antigen).

Flagellated bacteria have a characteristic number and location of flagella: some bacteria have one, while others have many. And according to these characteristics, mobile microbes can be divided into 4 groups:

**1. monotrichous** - bacteria having one flagellum at one pole of the cell (e.g. cholera vibrio);

**2. amphitrichous** - a bundle of flagella at both poles (e.g. Spirillum volutans);

**3. lophotrichous** - bacteria with a bundle of flagella at one pole (blue green milk bacillus);

**4. peritrichous** - bacteria that have flagella distributed over the entire surface of their bodies (e.g. E. coli, Salmonella).

**Spores** – are highly resistant structures which are formed in response to adverse conditions, by two genera of medically important gram-positive rods: the genus Bacillus, which includes the agent of anthrax, and the genus Clostridium, which includes the agents of tetanus, botulism, gas gangrene.

In bacilli and clostridia, spores are located:

**1. Centrally** (in the centre of the cell – B. anthracis);

**2. Terminally** (at the end of the rod – C. tetani);

**3. Subterminally** (towards the end – C. botulinum, C. perfringens) [2, 9,10].

**4. Illustrative material:** presentation material made in Power Point

**5. Literature:**

1. Medical Microbiology, Virology and Immunology. Part1. General Microbiology & Medical Immunology – Lecture Course for students of medical universities / I.I. Generalov. – Vitebsk, - VSMU. - 2016. - 282 p.

2. Borisov L.B. Medical Microbiology, Virology, immunology: Textbook. M.: 000 "Medical information Agency", 2001.

3. Pozdeev O.K. Medical Microbiology: textbook for universities. M.: GEOTAR - Media, 2007

4. http://www.berl.ru/article/small/bacter/principy_klaccifikacii_bakterii.htm

5. Korotyaev A.I., etc. of Medical Microbiology, immunology and Virology. Textbook for med. universities. – 2nd edition, Rev. – SPb; Spec. Lit., 2000.

6. http://www.e.lib.kemtipp.ru/uploads/32/tg089.doc

7. https://dic.academic.ru/dic.nsf/dic_microbiology/1119

8. Patrick R. Murray, PhD - Basic Medical Microbiology: textbook for universities.

9. Rajesh Bhatia, Rattan Lal Ichhpujani - Essentials of Medical Microbiology: textbook for universities. – 4th edition, Jaypee Brothers Medical Publishers *Editorial Consultant:* Ms. Peromila MA / English, 2008.

10. Apurba S Sastry, Sandhya Bhat, Anand Bhimaray J., Deepashree R- Essentials of Medical Microbiology: textbook for universities. – 3rd edition, Jaypee Brothers Medical Publishers, *The Health Sciences Publisher, New Delhi | London,* 2021.

**6. Checklist:**

1. Microbiology as a subject.

2. What are the historical periods in the formation and development of microbiology.

3. List the discovery of L. Pasteur.

4. Basic principles of classification of microorganisms.

5. What are the general properties of microorganisms.

6. What are the distinctive properties of cells prokaryotes and eukaryotes.

7. What 4 kingdoms are divided beings.

8. What are the specific characteristics of microorganisms used for their taxonomy and classification.

9. The principles of systematization of bacteria in the determinant, Bergi.

10. Classification of bacteria by type of food, give them a description.

## LECTURE № 2.

## 1. TOPIC: PHYSIOLOGY AND BIOCHEMISTRY OF BACTERIA AND VIRUSES

**2. Purpose:** To form concepts about the metabolism of bacteria and viruses in students. Explain the morphology, function and medical significance of bacteriophages.

**3. Abstracts of the lecture.**

The **physiology of bacteria** studies the vital activity of microbial cells – processes of their nutrition, respiration, growth, and reproduction.

**Metabolism** is a complex of biochemical pathways that give energy storage and synthesis of cellular structure. It consists of two highly related sets of reactions: catabolism and anabolism.

**Catabolism** (energy metabolism) is the process of decomposition of large molecules into simpler ones, leading to the accumulation of energy in the form of the driving force of protons, ATP or GTP.

**Anabolism** (synthesizing metabolism) provides the synthesis of macromolecules from which a cell is formed. It uses the energy accumulated as a result of catabolism. The metabolism of bacteria proceeds at a high rate and ensures the rapid adaptation of microorganisms to changing environmental situation.

All organisms, including microbes, can be classified metabolically according to their nutritional pattern – that is, their source of energy and their source of carbon and nitrogen. Considering the energy source bacteria generally classified as **phototrophs** or **chemotrophs**.

Bacteria which derive their energy from sunlight (solar energy) are called **Phototrophs**.

**Chemotrophs** gets energy from chemical reactions (oxidation-reduction reactions of inorganic or organic compounds).

For growth and multiplication of bacteria the minimum nutritional requirements are water, a source of carbon, a source of nitrogen and some inorganic salts. Water is the vehicle for the entry of all nutrients into the cell and for the elimination of all waste products. It participates in the metabolic reactions and also forms an integral part of the protoplasm.

Bacteria by their principal carbon and nitrogen source are subdivided into:

***Autotrophs*** (self-feeders): Some organisms may use very simple inorganic compounds, such as carbon dioxide as a carbon source and ammonium salts as a nitrogen source.

***Heterotrophs*** (feeding on others; GK-hetero-others) they are unable to synthesize their own metabolites and depend on pre-formed organic compounds. They require an organic carbon source, such as glucose, and obtain energy by oxidation or fermentation of organic substances. Often the same substance (for example, glucose) is used both as a carbon source and as an energy source. All bacteria inhabiting the human body fall into the heterotrophic group.

Autotrophs are also referred to lithotrophs (rock eating; GK-lithos-rock, stone; trophe-nutrition), and heterotrophs are referred to organotrophs. Based on the combination of the energy and carbon sources microrganisms are classified into:

**Photoautotrophs** – the source of energy is solar energy, the source of carbon and nitrogen are inorganic compounds-CO2, NO2.

**Photoheterotrophs** – the source of energy the same, the source of carbon and nitrogen are organic compounds.

**Chemoautotrophs** – they receive energy from the oxidation of inorganic substances, the source of carbon is CO2.

**Chemoheterotrophs** – the source of energy and carbon are organic compounds. Almost all of the medically important microorganisms are chemoheterotrophs. Typically, infectious organisms catabolize substances obtained from the host.

**Growth factors** – beside peptones, carbohydrates, fatty and inorganic elements bacteria require the special substances *growth factors*, which act as catalyst in the biochemical cellular processes and are structural units for the production of certain enzymes. Some microbes do not require a supplement of growth factors to the nutrient medium as they are able to synthesize these compounds themselves. These bacteria are named ***prototrophs***. Others grow poorly on growth factors free media; they are unable to synthesize growth factors themselves. These bacteria are named ***auxotrophs***. ***Growth factors are:*** vitamins, amino acids, purines, pyrimidines, lipids (cholesterol and other sterols), ferroporphyrins (Fe-porphyrins) [1, 8, 9, 10].

## MECHANISMS OF NUTRITION

The intake of nutrients into a bacterial cell is a complex of physico-chemical processes, and several factors contribute to these processes: different concentrations of nutrients, the size of molecules, their dissolution in water and lipids, the pH of the environment, penetration through membranes, etc.

There are **four mechanisms** of nutrient penetration into the cell:

**1. Passive diffusion.** Penetration of substances occurs due to the difference in nutrient concentrations inside and outside the cell. Energy expenditure is absent because it is realized from large concentration to the low. Water, oxygen, $CO_2$, penetrate by this mechanism.

**2. Light diffusion (facile).** Penetration occurs due to nutrient large concentration out of the cell. Membrane enzymes-translocases take part in these processes. At this time energy expenditure is absent, as the nutrients pass from the environment having high nutrient concentration to the one having low concentration (penetration over the gradient).

**3. Active diffusion.** It occurs when the nutrient concentration is low in the environment and the molecule transfer is realized from the environment having low concentration into the one having a high concentration (i.e. in the opposite

direction). Enzymes (permease) take part in these processes too. Due to this the active diffusion is accompanied with energy expenditure and the cell use ATP, which is accumulated by the oxidation – reduction reactions.

**4. Translocation of radicals.** Is an active process and it is accompanied with energy expenditure and enzymes participate in this process. Chemically changed molecules, that can't pass through the membrane in their unchanged structure, can also pass in the condition of active diffusion.

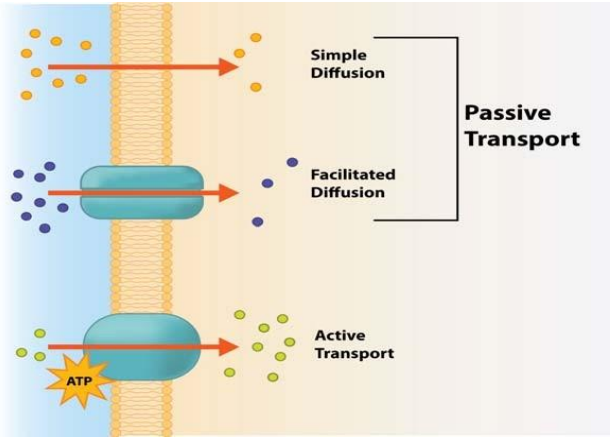


**Figure 2.1. The mechanisms of nutrition**

## BACTERIAL RESPIRATION

**Respiration in bacteria** is a complex process that is accompanied by the release of energy necessary for the microorganism to synthesize various organic compounds.

All microbes by type of respiration can be divided into (Figure 2.2):

**1. Obligate aerobes, w**hich will grow in the presence of oxygen, develop well in an atmosphere containing 21 per cent of oxygen. They require oxygen to grow because their ATP-generating system is dependent on oxygen as the hydrogen accepter. They grow on the surfaces of liquid (they form pellicle) and solid nutrient media (brucellae, tubercle bacilli, etc.).

**2. Facultative anaerobes.** They utilize oxygen to generate energy by respiration if it is present**,** and can be reproduced even in the absence of

molecular oxygen (the majority of pathogenic and saprophytic microbes). They may act in both ways.

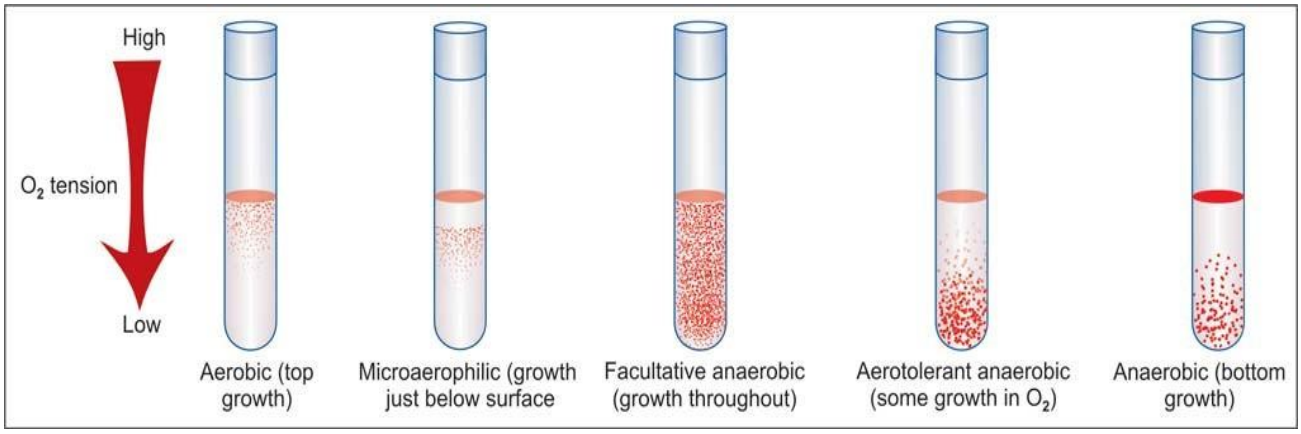


**Figure 2.2. Oxygen requirement of bacteria**

**3. Obligate anaerobes** for which the presence of molecular oxygen is a harmful growth-inhibiting factor, because they lack either superoxide dismutase or catalase, or both. Obligate anaerobes vary in their response to oxygen exposure; some can survive but are not able to grow, whereas others are killed rapidly, because of the result of accumulating of hydrogen peroxide. The catalase which splits hydrogen peroxide is absents in anaerobes and hydrogen peroxide kills the bacteria (causative agents of tetanus, botulism, gas gangrene anaerobic infections, etc.). Anaerobic bacteria use as electron acceptors compounds such as **nitrates** (facultative anaerobes-for example E coli) or **sulphates** instead of oxygen (anaerobic respiration).

**4. Microaerophiles** are microorganisms that can develop well in the presence of one percent oxygen.

**5. Aerotolerant organisms** such as Clostridium histolyticum; can survive for a short time, but not reproduce in oxygen conditions.

**6. Capnophilic** (Helicobacter) are microorganisms that are metabolically active (thrive) in the presence of high concentrations of carbon dioxide (CO2) (Figure 2.3).

**Figure 2.3. Candle jar**

## FACTORS AFFECTING BACTERIAL GROWTH

### Temperature

Most of the pathogenic bacteria grow optimally at 37°C (i.e. human body temperature). However, the optimal temperature range varies with different bacterial species. Accordingly, bacteria can be grouped into (Figure 2.4):

■ **Psychrophiles**: These grow best at temperatures below 20°C; example, most of the saprophytes, e.g. Pseudomonas;

■ ***Mesophiles***: These grow within a temperature range 25°C and 40°C; example, most of the pathogenic bacteria;

■ ***Thermophiles***: These bacteria grow at a high temperature range of 55°C–80°C, e.g. Geobacillus stearothermophilus [3, 10].

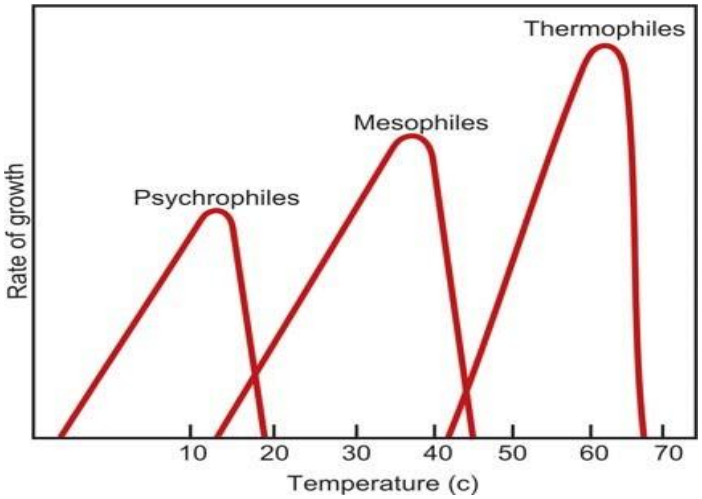


**Figure 2.4. Temperature requirement of bacteria**

**pH**

Most pathogenic bacteria growth between a pH of 7.2 and 7.6. precious few bacteria, such as lactobacilli, can growth at an acidic pH below 4.0. Many foods, such as pickles and cheese, are prevented from spoiling by acids formed during fermentation. V. cholerae is an example of bacteria that can grow in an alkaline environment pH [3, 10].

**Light**

Bacteria (except phototrophs) grow well in the dark. They are sensitive to ultraviolet rays and other light radiation. Photochromogenic mycobacteria produce pigments only when exposed to light [3].

**Osmotic pressure**

Bacteria are able to survive a wide range of external osmotic vibrations due to the mechanical strength of the cell wall. However, sudden exposure to hypertonic saline solution can cause cell shrinkage (plasmolysis), and exposure to distilled water can cause cell swelling and rupture (plasmoptysis) [3, 4, 10].

## ENZYMES AND THEIR ROLE IN METABOLISM

**An enzyme** is a cellular catalyst; it causes biochemical reactions to proceed many times faster than if they were not catalyzed. The participation of an enzyme can increase the reaction rate by millions or even billions of times. Their specificity is related to the active center formed by a group of amino acids. The names of enzymes usually end in–ase. The bacterial enzymes can be grouped into six classes; depending on the type of chemical reaction they catalyze (table2-1).

**Table 2-1 Enzyme classification based on type of chemical reaction catalyzed**

| CLASS | TYPE OF CHEMICAL REACTION CATALYZED | EXAMPLE |
|---|---|---|
| **1.Oxidoreductase** | Oxidation-reduction in which oxygenand hydrogen are gained or lost | **Cytochrome oxidase, lactate dehydrogenase** |
| **2. Transferase** | Transfer of functional groups such as an amino group, acetyl group, or phos-phate group | **Acetate kinase, alanine deaminase** |
| **3. Hydrolase** | Hydrolysis addition of water | **Lipase** |
| **4. Lyese** | Removal of groups of atoms withouthydrolyses | **Oxalate decarboxylase** |
| **5. Isomerase** | Re-arrangement of atoms within a Molecule | **Glucose-phosphate isomerase alanine racemase** |
| **6. Ligase** | Joining of two molecules using energyusually derived from breakdown of ATP | **Acetyl–CoA synthetase, DNA ligase** |

Bacterial enzymes are divided into the following groups:

- **Exo-enzymes** are released by the cell into the environment by splitting complex colloidal nutrients (hydrolase).
- **Endo-enzymes** are located inside the cell (catalase). Some of the endoenzymes in the bacterial cell act separately (monoenzymes); other enzymes are closely linked to each other (multienzymatic system) and provide a sequence of metabolic processes (enzymes of respiratory system).

According to the conditions of origin of enzymes, there are:

- **Constitutive enzymes** that are permanently present in the cell regardless of the presence of a catalyzing substrate (enzymes which take part in metabolic processes). These consist the main enzymes of cellular metabolism (lipase,

carbohydrase, oxidase, and catalase).

- **Adaptive enzymes** arise only in the presence of the appropriate substrate (β-lactamase, which breakdown penicillin, decarboxylase and etc.).

Bacteria have virulence by providing enzymes that promote the penetration of bacteria into the host cell and spread (hyaluronidase, neuraminidase, plasmocoagulase, fibrinolysin).

The functional activity of enzymes depends on environmental conditions (temperature, pH, substrate concentration). The rate of most chemical reactions increases as the temperature increases. Molecules move more slowly at low than at higher temperatures and may not have enough energy to cause a chemical reaction. Most enzymes have a pH optimum at which their activity is characteristically maximal. Enzyme activity and therefore the reaction rate declines above or below this pH value. Extreme changes in pH can cause denaturation. There is a maximum rate at which a certain amount of enzyme can catalyze a specific reaction. Only when the concentration of substrate(s) is extremely high can this maximum rate be attained [4, 9, 10].

## GROWTH AND MULTIPLICATION OF BACTERIA

Bacteria normally reproduce by ***binary fission***. The first step in division is cell elongation and duplication of the chromosomal DNA. The cell wall and cell membrane then begins to grow inward from all sides at a point between the two regions of the chromosomal DNA. Eventually, the in-growing cell walls meet, and two individual cells are formed, each of which is essentially identical to parent cells. Because one cell gives rise to two progeny cells, bacteria are said to undergo exponential growth (logarithmic growth).

The time required for a cell to divide (and its population to double) under optimum conditions is called the **generation time or population doubling time**. It varies considerably among organisms and with environmental

conditions such as temperature, the pH and other. Most medically important bacteria have generation time 20-30 minutes. Some bacteria are slow-growing; the generation time is 15- 48 hours (e.g. M. tuberculosis). When pathogenic bacteria multiply in host tissues, the situation may be intermediate between a **batch** cultures (nutrient are not renewed, nor are waste products removed. Under these conditions, the cell population increases in number in a predictable fashion and then eventually declines) and a **continuous** culture (nutrients must be continuously added and waste products removed); the source of nutrients may be inexhaustible but the parasite has to contend with the defense mechanisms of the body. Bacteria growing on solid media form colonies [2.3].

**Colony** is visible accumulation of generation of one mother cell on solid nutrient media. Each colony represents a clone of cells derived from single parent cell.

**Clone** is a population derived by binary fission from a single cell. Description of colonies by size, form, consistency, surface, pigments, the form of ends, transparency has differential diagnostic meaning.

The **growth** of microorganisms is an increase in the mass of bacterial cytoplasm as a result of the synthesis of cellular material. Bacteria generally **multiply** by the process of **binary fission (**in some cases by budding; viruses by reproduction). After a bacterial cell has increased in size and doubled all of its parts, it divides. One cell divides into two; those two divide to become four, and so on. In other words, the increase in cell numbers is exponential. DNA replication plays an important role in the process of mitotic binary fission of bacteria; the hydrogen bonds are ruptured and two DNA strands are formed, each one is contained in the daughter cells. The single stranded DNA is eventually linked by means of hydrogen bonds and again forms double-chain DNA responsible for genetic information.

In liquid media growth is diffuse. When a few bacteria are inoculated into a liquid growth medium and population is counted at intervals, it is possible to

plot a bacterial growth curve that shows the growth of cells over time [4, 5].

There are four basic phases of growth (Figure 2.4):

- **Lag phase:** when bacteria are seeded into fresh medium during which the number of cells are changed very little because the cells do not immediately reproduce in a new medium. During this period the organisms adapt themselves to growth in fresh medium and increase in size and metabolic activity (it can last for an hour or several days). During this time, however, the cells are not dormant. The microbial population is undergoing a period of intense metabolic activity involving, in particular, DNA and enzyme synthesis.
- **Log phase or exponential phase.** Eventually, the cells begin to divide and enter a period of growth multiplying at their maximum rate and their number is increased exponentially or by geometric progression with time. Cellular reproduction is most active during this period, and a generation time reaches a constant minimum. The log phase is the time when cells are most active metabolically. Beta- lactam drugs, such as penicillin, act during this phase because the drugs are effective when cells are making peptidoglycan, i.e., when they are dividing. Exponential phase is off limited duration because of: **1.** Nutrients exhaustion. **2.** Accumulation of toxic metabolic end products. **3.** Rise in cell density. **4.** Change in pH. **5.** Decrease in oxygen tension (in case of aerobic organisms).
- **Stationary phase:** this phase occurs when nutrient depletion or toxic products cause growth to slow until the number of new cells produced balances the number of cells that die, resulting in a steady state. Cells grown in a special apparatus called a thermostat**,** into which fresh nutrients are added and from which waste products are removed continuously, can remain in the log phase and do not enter the stationary phase.
- **Death phase (phase of decline) –** usually, the number of deaths soon exceeds the number of new cells formed, and the population enters the death or logarithmic decline phase. This phase continues until the population is

diminished to a tiny fraction of the number of cells in the previous phase, or the population might die out entirely. Finally, after a variable period, all the cells die and culture becomes sterile [7,9,10].

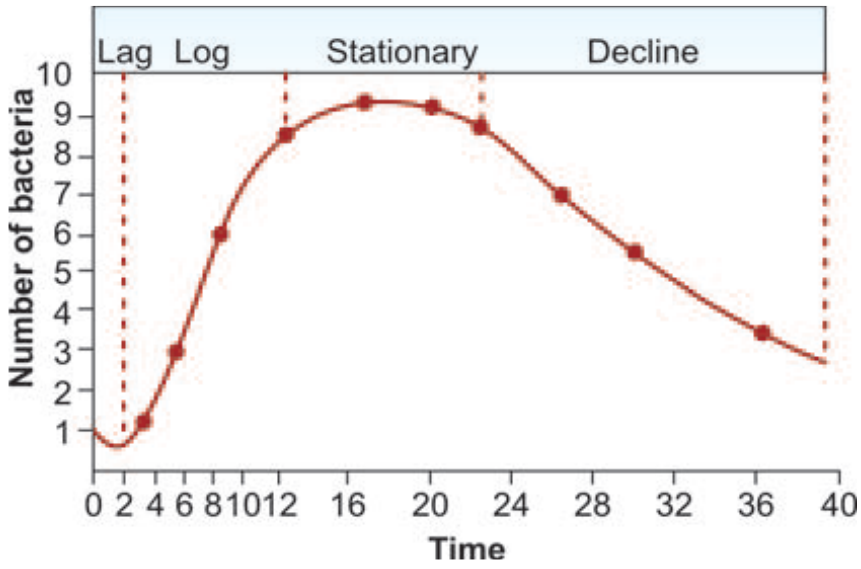


**Figure 2.4. Growth curve of viable bacteria**

## PRINCIPLES OF CULTIVATION AND IDENTIFICATION OF BACTERIA

Microorganisms (with the exception of obligate intracellular parasites — rickettsias, chlamydia, viruses and protozoa) are cultivated, as a rule, in synthetic nutrient media. According to the nutritional needs of a particular type, the nutrient medium must contain the relevant raw materials necessary for plastic and energy metabolism. The isolation of microorganisms from various materials and the production of their cultures is widely used in laboratory practice for the microbiological diagnosis of infectious diseases, in a scientific work and in the microbiological production of vaccines, antibiotics and other biologically active products of microbial activity.

The cultivation conditions also according to the properties of the relevant microorganisms. Most pathogenic microbes are cultivated on nutrient media at a

temperature of 37 ° C for 1-2 days. However, some of them require longer periods of time. For example, whooping cough bacteria — in 2-3 days, and Mycobacterium tuberculosis — in 3-4 weeks.

To stimulate the growth and reproduction of aerobic microbes, as well as to decline the time of their cultivation, the method of deep cultivation is used, which consists in continuous aeration and mixing of the nutrient medium. The deep method has found wide application in biotechnology. For the cultivation of anaerobes, special methods are used, the essence of which is to remove air or replace it with inert gases in sealed thermostats - anaerostats.

Anaerobes are grown on nutrient media containing reducing substances (glucose, formic sodium, etc.) that reduce the redox potential. In diagnostic practice, pure cultures of bacteria that are isolated from the material under study taken from the patient or the environment are of particular importance.

For this purpose, artificial nutrient media are used, which are subdivided into basic, differential diagnostic, and elective of the most diverse composition. The choice of a nutrient medium for the isolation of a pure culture is important for bacteriological diagnostics. In most cases, solid nutrient media, previously poured into Petri dishes, are used. The study material put on the surface of the medium using a loop and rubbed with a spatula to obtain isolated colonies grown from a single cell. Subculture of an isolated colony on a canted agar medium into a tube results in a pure culture.

For identification, i.e. determining the genus and species of the selected culture, most often studying phenotypic traits:

a) the morphology of bacterial cells in stained smears or native preparations;

b) biochemical characteristics of the culture according to its ability to ferment carbohydrates (glucose, lactose, sucrose, maltose, mannitol, etc.),

c) to form indole, ammonia and hydrogen sulfide, which are products of the proteolytic activity of bacteria.

For a more complete analysis, gas-liquid chromography and other methods are used. Along with bacteriological methods for the identification of pure cultures, immunological methods are widely used, which are aimed at studying the antigenic structure of the selected culture.

For this purpose, serological reactions are used: agglutanation, precipitation of immunofluorescence, complement fixation, enzyme immunoassay, radio-immune methods, etc. [5].

## PHYSIOLOGY OF VIRUSES

Viruses are obligate intracellular parasites capable only of intracellular reproduction. In a virus-infected cell, viruses may be present in various states:

• reproduction of numerous new virions;

• the presence of the virus nucleic acid in the integrated state with the chromosome of the cell (in the form of a provirus);

• existence in the cytoplasm of a cell in the form of circular nucleic acids resembling bacterial plasmids.

Therefore, the spectrum of disorders caused by the virus is very wide: from severe productive infection ending in cell death to prolonged interaction of the virus with the cell in the form of latent infection or malignant transformation of the cell. There are three types of virus-cell interaction*: productive, abortive*, and *integrative*.

1. ***Productive type*** - ends with the formation of a newly generation of virions and the death (lysis) of infected cells (cytolytic form). Some viruses leave the cells without destroying them (non-cytolytic form).

2. ***Abortive type*** - does not finish with the formation of new virions, since the infectious process in the cell is aborted at one of the stages.

3. ***Integrative type, or virogeny*** - is characterized by the inclusion (integration) of viral DNA in the form of a provirus into the chromosome of the cell and their coexistence together (joint replication).

3.3.1. ***Reproduction of viruses.*** The productive type of interaction of the virus with the cell, i.e., the reproduction of the virus (lat. Ge - repetition, productio - production), occurs in 6 stages:

1) virion adsorption on the cell;

2) virus penetration into the cell;

3) "stripping" and release of the viral genome (deproteinization of the virus);

4) synthesis of viral elements;

5) formation of virions;

6) release of virions from the cell. In different viruses, these stages occur in different ways.

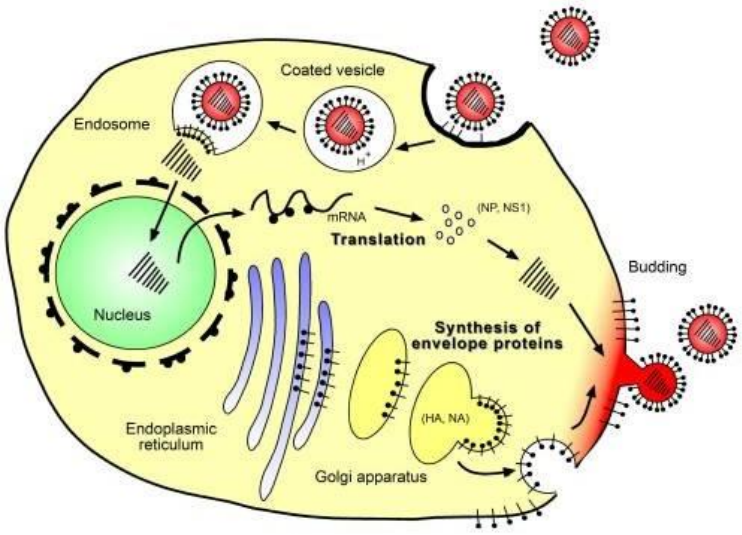


**Figure 2.4. Interaction of the virus with the host cell**

**Adsorption of viruses.** The first stage of viral reproduction is adsorption, i.e., attachment of the virion to the cell surface. It takes place in two phases.

The first phase is non-specific, due to the ionic attraction between the virus and the cell, consist of other mechanisms.

The second phase of adsorption is highly specific, due to the homology and complementarity of the receptors of sensitive cells and the viruses “recognizing” their protein ligands.

Proteins on the surface of viruses that recognize specific cellular receptors and interact with them are called attachment proteins (mainly glycoproteins) inside the lipoprotein membrane. Specific cell receptors have a different nature, being proteins, lipids, carbohydrate components of proteins, lipids, etc. Thus, the receptor of the fippa virus is sialic acid in glycoproteins and glycolipids (gangliosides) of respiratory tract cells. Rabies viruses are adsorbed on acetylcholine receptors of nervous tissue, and the human immunodeficiency viruses are adsorbed on CD4 receptors of T-helper cells, monocytes and dendritic cells. On one cell is from ten to one hundred thousand specific receptors, so tens and hundreds of virions can be adsorbed on it.

The presence of specific receptors underlies the selectivity of viruses that infect certain cells, tissues and organs. This is the so-called ***tropism*** (Greek: tropos - turn, direction). For example, viruses that multiply mainly in liver cells are called hepatotropic, in nerve cells, neurotropic, in immunocompetent cells, immunotropic, etc. [10].

**The penetration of the virus into the cell.** Viruses enter the cell by receptor-dependent endocytosis (viropexis), or by fusion of the virus envelope with the cell membrane, or as a result of a combination of these mechanisms.

1. Receptor-dependent endocytosis occurs as a result of virion capture and absorption by the cell: a cell membrane with an attached virion is implanted to form an intracellular vacuole (endosome) containing a virus. Due to the ATP-dependent "proton" pump, the contents of the endosome are acidified, which leads to the fusion of the lipoprotein envelope of the complex virus with the endosome membrane and the release of the viral nucleocapsid into the cytosol of the cell. Endosomes connect with lysosomes, which destroy the remaining viral

components. The process of isolation of less (simply organized) viruses from the endosome into the cytosol remains poorly understood.

2. Fusion of the virion shell with the cell membrane is characteristic only of some viruses with a shell (paramyxoviruses, retroviruses, herpes viruses), that contain fused proteins. There is a point interaction of the viral fusion protein with the lipids of the cell membrane, as a result of which the viral lipoprotein envelope integrates with the cell membrane, and the internal component of the virus enters the cytosol of the cell [6, 8, 9].

## INDICATION OF VIRUSES AND VIRUS ISOLATION

Indication of viruses and isolation of viruses (as a more private section of this line of research) are the main components of the virological diagnosis of viral infections. Detection of viruses, viral antigens, viral RNA or DNA, and in some cases, characteristic pathological changes (for example, in the liver with yellow fever) or specific inclusions (for example, Babesh-Negri body in brain tissue with rabies) provide the necessary information to solve many questions. The obtained data helps to decipher the etiology of the disease, establish the circulation of viruses in the areas surveyed, study many cardinal aspects of the epidemiology and epidemiology of zoonotic viral infections, determine the sanitary condition of the territory in relation to some anthropogenic infections caused by, for example, widespread enteroviruses [7].

Newborn and adult white mice, guinea pigs, white rats, Syrian hamsters, monkeys, chicken and duck embryos, various types of transplanted cell cultures: Syrian hamster buds, piglets, monkeys, dogs are used as laboratory models for isolating viruses from clinical and field materials, primary cultures of fibroblasts of human embryos, chickens and many others. In sensitive species of laboratory animals infected with virus-containing material, there is a clinically pronounced disease and death. The brain, and sometimes other organs and tissues of sick

animals are a good source of preparation of specific viral antigens. The result of infection with the virus-containing material of cell cultures can be the development of cytopathic action, the formation of plaques under the agar coating, the phenomenon of an interfering action detected by repeated inoculation of cells with an indicator, obviously cytopathogenic virus [8].

The method of infection of mosquitoes, primarily large non-blood sucking mosquitoes of the genus Toxorhynchites, has high efficiency in isolating dengue, some other flaviviruses and bunyaviruses.

In order to indicate viruses in clinical, field and experimental materials, highly sensitive serological methods are used using poly- or monoclonal antibodies labeled with enzymes (for example, horseradish peroxidase, alkaline phosphatase, etc.), fluorochromes, radioactive nuclides and lanthanides. Of these, the most widely used are ELISA, indirect MFA, and to a lesser extent RNA and lanthanide immunofluorescence analysis (LIFA).

Modifications of the polymerase chain reaction method, RT-PCR and time are widely used for the detection of viral nucleic acids. The method of molecular hybridization of nucleic acids has not lost its importance today.

The following materials are used to isolate and detect viruses from clinical and sectional materials:

1) for diseases of the respiratory system, measles, rubella: smears from the pharynx, sputum, feces, pieces of lung, scrapings from the bronchi;

2) for diseases of the central nervous system:

- rabies: saliva, brain;
- American horses encephalitis: blood, CSF, pharyngeal washes;
- tick-borne encephalitis, Japanese encephalitis, etc.: blood, CSF, brain;
- Herpes: pharyngeal washes, CSF, brain;
- ECHO infections and Coxsackie infections: pharyngeal washes, CSF, feces, brain;

3) in diseases of the skin and mucous membranes - measles, rubella, herpes simplex, smallpox, and smallpox diseases - pharyngeal washes; with herpes simplex, chicken pox and shingles - the vesicle fluid is additionally taken;

4) for hemorrhagic fevers - yellow hemorrhagic fever, Crimean hemorrhagic fever, Ebola hemorrhagic fever, etc. - blood, internal organs, and sometimes brain tissue [8, 9, 10].

**4. Illustrative material:** a media projector.

**5. Literature:**

1. Medical Microbiology, Virology and Immunology. Part 1. General Microbiology & Medical Immunology – Lecture Course for students of medical universities / I.I. Generalov. – Vitebsk, - VSMU. - 2016. - 282 p.

2. Rajesh Bhatia, Rattan Lal Ichhpujani - Essentials of Medical Microbiology: textbook for universities. – 4th edition, Jaypee Brothers Medical Publishers *Editorial Consultant:* Ms Peromila MA (English), 2008

3. Subhash Chandra Parija - Textbook of Microbiology and Immunology - 2nd Edition, Published by Elsevier, a division of Reed Elsevier India Private Limited: Elsevier, 2012

4. Satish Gupte – The Short Textbook of Medical Microbiology (Including Parasitology): – 10th edition, Jaypee Brothers Medical Publishers (P) Ltd: New Delhi, 2010.

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6. Borisov L. B. Medical Microbiology, Virology, immunology: Textbook. M.: 000 "Medical information Agency", 2001.

7. Medical Microbiology, Virology and Immunology. Part 2. Medical Bacteriology & Medical Virology – Lecture Course for students of medical universities / I.I. Generalov. – Vitebsk, - VSMU. - 2016. - 392 p.

8. Pozdeev O. K. Medical Microbiology: textbook for universities. M.: GEOTAR - Media, 2007

9. Korotyaev, A. I., etc. of Medical Microbiology, immunology and Virology. Textbook for med, schools. – 2nd edition, Rev. – SPb. Spec. Lit., 2000.

10. Apurba S Sastry, Sandhya Bhat, Anand Bhimaray J., Deepashree R- Essentials of Medical Microbiology: textbook for universities. – 3rd edition, Jaypee Brothers Medical Publishers, *The Health Sciences Publisher, New Delhi | London,* 2021.

**6. Checklist:**

1. The metabolism of bacteria.
2. The chemical composition of bacterial cells.
3. What are the factors of bacterial growth.
4. What are the main pathways of metabolites and ions in the microbial cell.
5. What enzymes are synthesized by microorganisms?
6. How many phases have growth curve?
7. Principles of cultivation and identification of bacteria
8. Types of virus-cell interactions.
9. Adsorption of viruses.
10. Indication of viruses.

## LECTURE № 3.

## 1. TOPIC: FUNDAMENTALS OF CHEMOTHERAPY FOR INFECTIOUS DISEASES, ANTIBIOTICS, STRATEGY OF ANTIBACTERIAL THERAPY

**2. Purpose:** To acquaint students with the classification of antibiotics. Disassemble the mechanisms of antimicrobial action of antibiotics. Introduce antibiotic susceptibility testing methods.

**3. Abstracts of the lecture.**

Different antimicrobial substances that influence pathogenic microorganisms are widely applied for the treatment of patients with infectious diseases, and in some cases for the prevention of diseases.

**Antimicrobial chemotherapy** is the treatment of bacterial, viral, fungal and protozoan infections with chemical antimicrobial drugs.

The safety and efficacy of any antimicrobial drug can be described by its **therapeutic index** (also known as *therapeutic ratio* or *therapeutic window*).

This is the most likely pronounced as the highest dose that the patient can tolerate without toxic effects **separated by** the dose needed to control the infection (hence, provides the desired efficacy).

The chemical preparation is suitable for medical use if its therapeutic ratio is ***at least than 3***.

Antimicrobial drugs, which are used for treatment and prevention of infections in humans and animals, are divided into two main groups – ***antiseptics*** and ***antibiotics*** [1].

Antibiotics (anti-against, bios-life) are chemicals secreted by some microorganisms that inhibit the growth and development of other microbes, which is based on antagonistic relationships between microorganisms of different species.

The study of antibiotics started in 1929, when A. Flaming proved that the filtrate of a broth culture of the fungus Penicillium notatum has antibacterial

properties.

The further development of this problem is connected with the work of various scientists: R. Dubos isolated gramicidin and thyrocidin from the cultural liquid of *S. brevis*; S. Waksman and his colleagues developed a method of producing streptomycin. B. Tokin discovered antimicrobial substances from plants — phytoncides and others, which complete modern medical practice with numerous drugs widely used in medicine. It is used for the treatment of infectious diseases.

Antibiotics must comply with several requirements:

1. High antimicrobial activity and selectivity in doses that are non-toxic to the patient.

2. Efficient therapeutic influence on tissues and organs, low level of inactivation by tissue proteins and enzymes.

3. Absence or slow development of side effects.

4. A long period of metabolism (prolonged effect).

5. Slow growth of resistance of microorganisms to the antibiotic.

6. High efficacy of the drug at a low cost of therapy.

7. The preparation should be suitable for various practical applications and stable during storage.

Unfortunately, none of the known antibiotics fully covers all these requirements [1].

Antimicrobial drugs vary in their spectrum of activities. They may be broad-spectrum or narrow-spectrum antibiotics.

1. ***Broad-spectrum or extended-spectrum antibiotics*** are active against a wider range of different microbes. For example, tetracyclines are active against various Gram-positive and Gram-negative bacteria, rickettsiae, mycoplasmas, and even protozoa.

2. ***Narrow-spectrum antibiotics*** are effective against one or very few microbes. For example, vancomycin is active against certain Gram-positive

bacteria (such as staphylococci and enterococci) or griseofulvin, which is used only against fungal skin infections.

Antimicrobial drugs can be ***bactericidal*** or ***bacteriostatic***. **The bactericidal drug** kills bacteria, whereas **the bacteriostatic drug** suppresses the growth of bacteria, but does not kill them.

## CLASSIFICATION OF ANTIBIOTICS

Antibiotics are classified according to the:

❖ **Chemical structure of the drug:**

1**. *β-lactamates***

- penicillin
- cephalosporin
- cycloserine

2. ***Poliens*** – nystatin, levorin, amphotericin B, etc.

3. ***Polymyxins*** – polymyxin A, polymyxin B

4. ***Aminoglicozides*** – streptomycin, neomycin, monomycin, kanamycin, gentamycin, etc.

5. ***Tetracycline*** – tetracycline, oxytetracyclin, chlortetracycline, rondomycine, etc.

6. ***Macrolides*** – erythromycin, oleandamycine

7. ***Rifamicin*** – rifamicin, rifampicin

❖ **Origin:** Antibiotics produced by

1. ***fungi*** – Penicilium and Cephalosporium are used for this purpose.

2. ***actinomycetes*** – frequently genus Streptomyces (streptomycin), is used which has a good therapeutic action on *M. tuberculosis*, *F. tularensis*, on causative agent of whooping cough, etc. Erythromycin, nystatin are the products of Streptomycin. 80 percent of antibiotics are produced by Actinomycetes.

3. ***bacteria*** – for this purpose genus Bacillus and genus Pseudomonas are used (polymixins are prepared from bacteria).

4. ***tissues*** (animal origin): *lysozyme*, which discovered in saliva, tear. Lysozyme has bactericidal action on bacteria they are break the glycoside bonds in bacterial cell wall. The next antibiotics are *ecmoline, ectericid,* which are synthesized by fish tissues. *Interferons* (α, β) are glycoproteins produced by human and other animal cells after viral infection. They inhibit the reproduction of viruses by blocking the translation of viral proteins. Interferons are universal, are not virus specific (IFN are active against all viruses).

5. ***plants*** – volatile plant substances phytoncides have bactericidal properties, which cause a lethal effect and they are used for treatment of infectious diseases. Onion, garlic, tomato are contain volatile and are bactericidal products.

❖ **The way of reception:**

1. **biological synthesizes** – (natural antibiotics) during this method high productive stem is used which is cultivated on special media (penicillin).

2. **chemical** – synthesizing of antibiotics from chemical substances (synthetic antibiotics-levorin).

3. **combined (complex) method:** here two methods are used: complex of biological and chemical methods. By the biological method the main ring of antibiotic is obtained than chemicals are added and the new preparation is prepared (e.g. cephalosporin, semisynthetic penicillin). These drugs are used in the treatment of diseases caused by penicillin-resistant staphylococci and other pathogens.

**Mechanisms of action of antibiotics and antimicrobial spectrum**

1. **Antibacterial antibiotics.** The numerous groups. In this group antibiotics subdivided are into broad (large) spectrum and narrow spectrum. Antibiotics of broad spectrum act on the whole groups of bacteria: gram negative, gram-positive (Gracilicutes, Firmicutes, Tenericutes). Narrow 3 spectrum antibiotics acted on the few (small) groups of bacteria (e.g. only gram negative or only gram positive).

2. **Antifungal antibiotics.** There are few preparations in this group - nystatin, levorin the drugs against genus Candida. Amphotericin B, which has large spectrum and act on Candida genus and the other genesis (e. g. Blastomycosis, Aspergiliuosis, etc.)

3. **Antiprotozoal group.** There are few antibiotics in this group in major narrow spectrum (Fumagilin).

4. **Antiviral group:** It is the very difficult of chemotherapy of viral diseases. At present there are no effective drugs against viral infections. This is due to the biological features of viruses as obligate intracellular parasites [1, 3].

## SIDE EFFECTS OF ANTIBIOTICS ON MACROORGANISMS (DANGERS OF INDISCRIMINATE USE)

**1. Direct drug toxicity.** The toxic action is associated with the quality of preparation, doses, with the kind of employment and the state of patients. Antibiotics can be hepatotoxic (tetracyclines), nephrotoxic (aminoglycosides), ototoxic (streptomycin, gentamycin). It has been installed that high doses of penicillin and streptomycin provide a neurotoxic action. Levomycetin has toxic effect on the haematopoetic organs. Cephalosporins suppress the synthesis of vitamins and can cause bleeding. Antibiotics has a toxic effect on fetus - *teratotoxic action* (anzamycin, levomycetin)

**2. Changes in the normal flora of the body.** Antimicrobial drugs affect not only the infecting microorganisms but also susceptible members of the normal microflora of the body. An imbalance appears which cause profound disturbances of symbiotic relationships among normal microflora resulting in disbacteriosis. The condition of disbacteriosis enhances intensive multiplication and spread of some of the co members of the intestinal, mucosal and skin biocoenosis and their transformation from a saprophyte state into conditionally pathogenic and pathogenic forms. As a result local and general lesions develop. The oppressing of

normal flora with antibiotics resulting in the oppressing of antagonism and it promote the development of infectious disease, or development of secondary infections. To prevent these complications we use the following methods:

1) If it is possible using antibiotics of narrow spectrum,

2) With antibiotics it is required to use antifungal preparations,

3) To restore the normal microflora it is necessary to prescribe eubiotics.

**3. Influence on immune system:** Widespread sensitization of the population leads to *hypersensitivity*, anaphylaxis, rash, fever, blood disorders, cholestatic hepatitis and collagenvascular diseases. The occurrence of these reactions depends on the quality of preparation, the way of injection, from the individual felt of patient. Quite frequently allergic reactions arise during local application of antibiotics. During these reactions can develop allergic rash, contact dermatitis, anaphylactic reactions. In order to avoid the allergic reactions before the using of antibiotics Bezredka test is used.

Immunodepression can occur during antibiotic therapy; this can be used in transplantation (e.g., cyclosporine which is produced as an antifungal preparation, but immune depressive function is higher and now it used in transplantation).

Bacteria and bacterial cell can lose the main antigenic structures during antibiotic therapy and due to this cannot be presented complete antigenic functions, due to this, immune response is not complete and infection can transform into chronic form, relapse or re-infection.

To prevent these complications immune- antibiotic- therapy can be used. It means parallel using of antibiotics and vaccines. Under the action of antibiotics microorganism is killed, under the action of vaccines immune system is stimulated.

## SIDE EFFECTS OF ANTIBIOTICS ON MICROORGANISM

During antibiotic therapy changes in structure of the bacterial cell, in biochemical activity (e.g., L-forms can form. It leads to difficulties in diagnosis) can occur.

**During antibiotic therapy resistance to antimicrobial drugs can occur.** There are many different mechanisms by which microorganisms might exhibit resistance to drugs: Innate and acquired. Example of innate resistance is mycoplasma. This bacteria is without cell wall (it is innate property), and penicillin cannot act on this bacteria.

Acquired drug resistance is subdivided into genetic (chromosomal) and biochemical (extrachromosomal) resistance.

**Genetic origin of drug resistance:** Most drug resistant microbes emerge as a result of genetic change and subsequent selection processes by antimicrobial drugs.

**Chromosomal (genetic) resistance:** This develops as a result of spontaneous mutation in a locus that controls susceptibility to a given antimicrobial drug. Chromosomal resistance to antibiotic develops only to single drug.

**Extrachromosomal resistance:** Bacteria often contain extrachromosomal genetic elements called plasmids (e.g., R-factor). R factors are a class of plasmids that carry genes for resistance to one- and often several-antimicrobial drugs. Antimicrobial resistance of plasmid genes often controls the formation of enzymes capable of breaking antimicrobials.

Thus, plasmids determine resistance to penicillin and cephalosporin by carrying genes for the formation of β-lactamases. Microorganisms produce enzymes that destroy the drug. Examples: Staphylococci are resistant to penicillin by producing β-lactamase enzyme that destroys the drug.

Genetic material and plasmids can be transferred by the transduction, transformation, and conjugation.

Besides, changes in permeability of membranes (against tetracycline), changes in target cells (e.g., during streptomycin therapy changes in ribosomal proteins on which this drug is binds occur).

There are three cases by which antibiotic can leave bactericidal or bacteriostatic action:

1. Antibacterial drug must enter into the cell,
2. Antibacterial drug must interact with target cell,
3. Antibiotic during this interaction must save primary structure [4].

**Basics of rational antibiotic therapy.** The prevention of the development of complications consists primarily in the observance of the principles of rational antibiotic therapy (antimicrobial chemotherapy):

• ***Microbiological principle***. It is necessary to formulate a specific etiological diagnosis. The antibiotic should be used only during an infection in which a microorganism is involved. It is necessary to isolate a pure culture; it is preferable to obtain a representative sample before the introduction of antimicrobials. Before prescribing this drug, it is necessary to measure antimicrobial activity. To measure antimicrobial activity, we will use two main methods: dilution or diffusion.

• ***Pharmacological principle***. Before giving antimicrobial drug it is necessary to define correct dose, the intervals, route of injection. It is necessary to know drug combinations.

• ***Clinical principle***. Before giving these drugs it is necessary to take into consideration the state of the patient, age, sex, the condition of immune status, pregnancy, attendant diseases.

• ***Epidemiological principle***. The choice of the drug, especially for inpatients, must take into account the state of resistance of the microbial strains circulating in a given department, hospital, and even the region. It should be remembered that antibiotic resistance can not only be acquired but also lost, while restoring the microorganism's natural sensitivity to the drug. Only natural resistance does not change.

• ***Pharmaceutical principle***. It is necessary to consider the expiration date and comply with the rules1 storage of the drug, since if these rules are violated, the antibiotic can not only lose its activity, but also become toxic due to degradation. The cost of the drug is also important [5].

Currently, in clinical practice, there are two principles, the appointment of antibacterial drugs: an empirical and etiotropic. **Empirical antibiotics** are based on knowledge about the natural sensitivity of bacteria, epidemiological data on the resistance of microorganisms in the region or hospital, as well as on the results of controlled clinical trials. The obvious advantage of the empiric setting of chemotherapy drugs is the possibility of fast initiation of therapy. In addition, this approach excludes the costs of conducting additional research.

Empirical antibiotics based on knowledge about the natural sensitivity of bacteria, epidemiological data on microbial resistance in the region or hospital and the results of controlled clinical trials

However, the ineffectiveness of antibiotic therapy, for nosocomial infections, which are difficult to predict a causative agent and its sensitivity to antibiotics seek to establish a causal treatment. **Etiotropic antibiotics** involves not only the allocation of the pathogen from clinical material, but also to determine its sensitivity to antibiotics. The obtaining of correct data is possible only under condition of competent performance of all parts of the bacteriological examination: from the capture of clinical material, its transportation to the bacteriological laboratory identification of the pathogen to determine its sensitivity to antibiotics and interpreting the results.

Etiotropic antibiotics are based on isolating the causative agents from the source of infection and determining its sensitivity to antibiotics.

The second reason necessitating the determination of sensitivity of microorganisms to antimicrobial drugs is obtaining epidemiological data on the structure of resistance of causative agents of community-acquired and nosocomial

infections. In practice, these data are used in the empirical prescription of antibiotics, as well as for the formation of hospital forms.

**Methods of determining sensitivity to antibiotics**

Methods of determining the sensitivity of bacteria to antibiotics are divided into 2 groups: diffusion and dilution methods.

**The definition of sensitivity of bacteria to antibiotics:**

***diffusion methods***

• using discs with antibiotics

• using E-tests

***breeding methods***

• cultivation in liquid nutrient medium (broth)

dilution in agar

When determining the sensitivity of the disk diffusion method, a bacterial suspension of a certain density (usually equivalent to 0.5 McFarland standard turbidity) is applied to the surface of the agar in a Petri dish, and then disks containing a certain amount of antibiotic are placed. The diffusion of the antibiotic into the agar leads to the formation of a zone of suppression of the growth of microorganisms around the discs.

After incubation cups in a thermostat at a temperature 35°-37°C during the night, consider the result by measuring the zone diameter around the disc in millimeters [5].

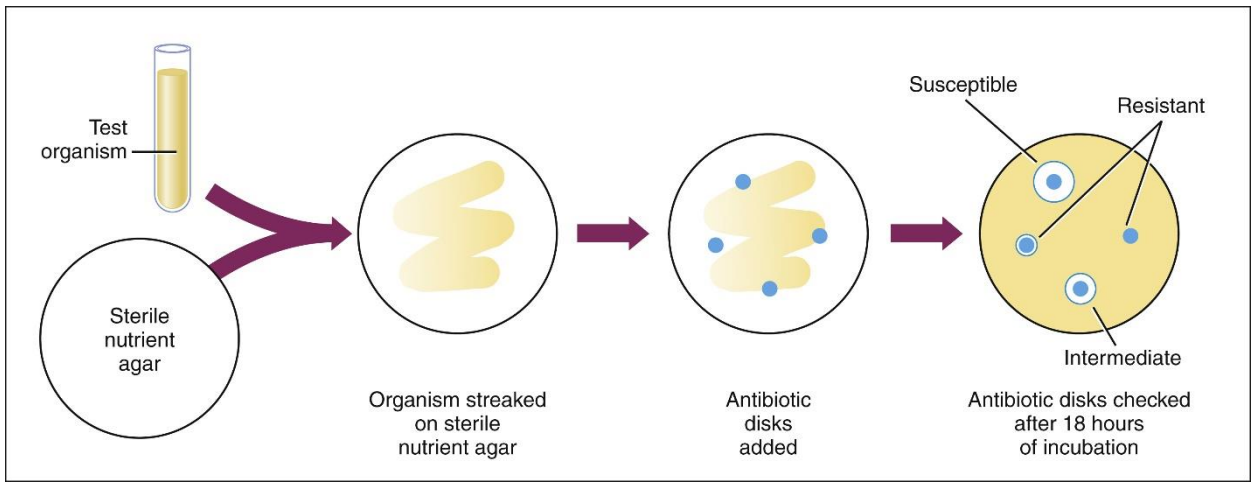


**Figure 3.1. The definition of sensitivity of microorganisms disco-diffusion method.**

The definition of sensitivity of a microorganism using the ***E-test*** is like testing the disk diffusion method. The difference is that instead of the disk with antibiotic use strip E-test containing concentration gradient of antibiotic from the maximum to the minimum. At the intersection of the elliptical zone of growth inhibition with the strip E-test have the value of the minimal inhibitory concentration (MIC).

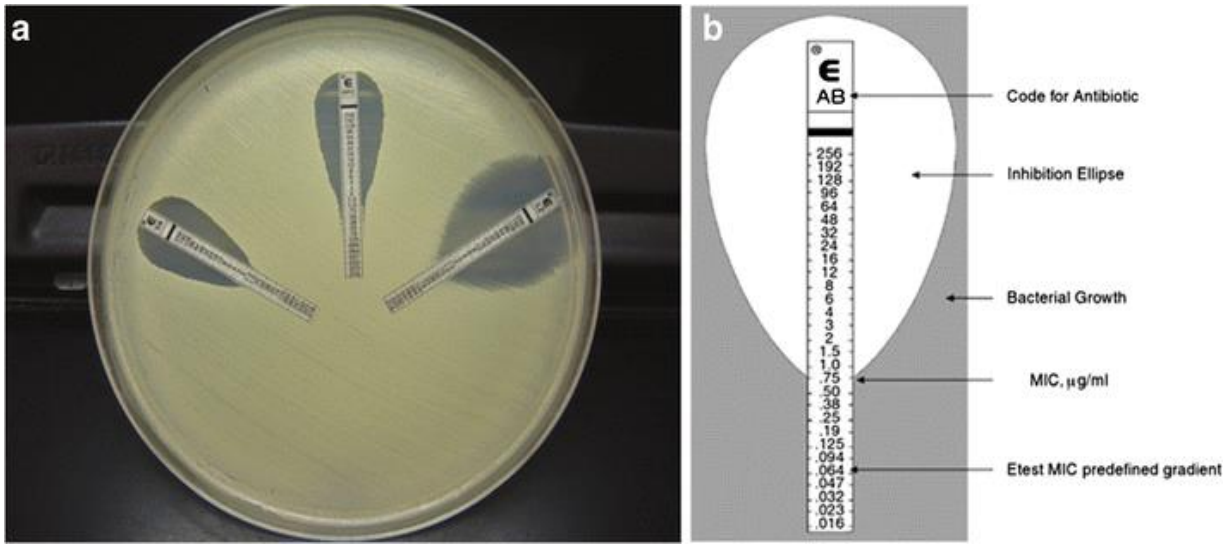


**Figure 3.2. The definition of sensitivity of microorganisms using E-tests.**

The undoubted advantage of diffusion methods is the ease of testing and availability of execution at any bacteriological laboratory. However, given the high cost of E-test for routine work typically used disk diffusion method [2, 8].

Breeding methods based on use of double serial dilutions of concentrations of antibiotic from the maximum to the minimum (for example from 128 µg/ml, 64 µg/ml, etc. up to 0.5 µg/ml, 0,25 mg/ml and 0.125 µg/ml). At the same antibiotic at various concentrations contribute in a liquid nutrient medium (broth) or agar. Then a bacterial suspension of a certain density corresponding to the standard turbidity of 0.5 according to McFarland is placed in a broth with an antibiotic or on the surface of the agar in a cup. After incubation overnight at a temperature of 35 ° C-37 ° C is carried out in the light of the results obtained. The presence of microbial growth in the broth (turbidity of the broth) or on the surface of the agar indicates

that the concentration of the antibiotic is sufficient to suppress its viability. With increasing concentration of the antibiotic, the growth of a microorganism deteriorating. First the lowest concentration of antibiotic (from the series of consecutive dilutions), where visually is not determined by bacterial growth is considered to be the **minimal inhibitory concentration (MIC**). MIC is measured in mg/l or µg/ml.

Minimum overwhelming concentration (MOC) - the lowest concentration of antibiotic (mg/l or µg/ml), which in vitro completely inhibits visible bacterial growth.

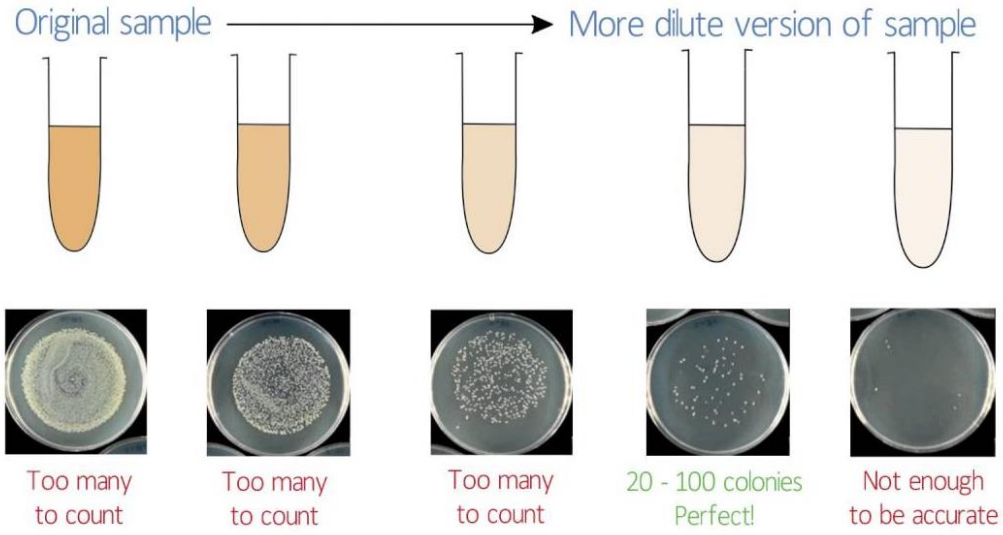


**Figure 3.3. Determine the value of the IPC by the method of dilutions in liquid nutrient medium.**

### Interpretation of results of susceptibility

Based on the obtained quantitative data (the diameter of the antibiotic growth inhibition zone or the MIC value), microorganisms are divided into sensitive, moderately resistant and resistant (Figure 3.4). To distinguish these three categories of sensitivity (or resistance) between use so-called **edge concentration** (breakpoint) antibiotic (or boundary values of the zone diameter of growth inhibition of the microorganism).

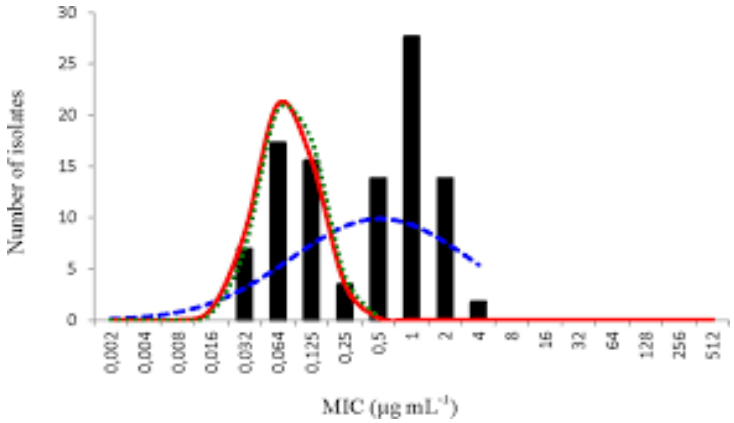


**Figure 3.4. Interpretation of results determination of sensitivity of bacteria in accordance with the values of the MIC.**

Boundary concentrations are not constant values. They can be reviewed, depending on changes in the sensitivity of a population of microorganisms. Development and revision of criteria of interpretation engaged in by the leading specialists (chemiotherapeutic and microbiologists) included in special committees. One of these is the national Committee for clinical laboratory standards (National Committee for Clinical Laboratory Standards - NCCLS). Currently, NCCLS standards are acknowledged worldwide and are utilized internationally valuation the results of determining the sensitivity of bacteria in multicenter microbiological and clinical studies.

There are two methods of interpreting the results of sensitivity determination - microbiological and clinical. The microbiological interpretation is based on the analysis of the separation of concentrations of antibiotics that inhibit the viability of bacteria. The clinical interpretation is based on the study of the effectiveness of antibacterial therapy.

**Sensitive microorganisms (susceptible)**

Clinically, they are classified as sensitive bacteria (with parameters obtained in vitro) if a good therapeutic effect is observed when treating infections caused by these microorganisms with standard doses of an antibiotic.

In the absence of reliable clinical information, the unit for determining the sensitivity category is based on a joint study of in vitro data and pharmacokinetics,

i.e. on the concentration of the antibiotic achievable at the site of infection (or serum).

**Resistant microorganisms (resistant)**

Resistant (resistant) bacteria are referred to when there is no effect from therapy in the treatment of infections caused by these organisms, even when using maximum doses of an antibiotic. Such microorganisms have resistance mechanisms.

**Microorganisms with intermediate resistance (intermediate)**

Clinically, intermediate resistance in bacteria occurs if an infection caused by these strains may have a different therapeutic result. However, treatment can be successful if the antibiotic is used in a dosage higher than the standard, or the infection is localized in the place where the antibacterial drug accumulates in high concentrations.

From a microbiological point of view, bacteria with intermediate resistance are subpopulation corresponding to MIC values or the diameter of the zones between sensitive and resistant microorganisms. Sometimes cultures with medium resistance and resistant bacteria are combined into one category of resistant organisms.

It should be noted that the clinical interpretation of the sensitivity of bacteria to antibiotics is conditional, since the outcome of therapy does not always depend only on the activity of the antibacterial drug against the pathogen. Clinicians have known cases where for microbial resistance, according to in vitro studies, received a good clinical effect. Conversely, when susceptibility may be the ineffectiveness of therapy.

In certain clinical situations when there are insufficient results on the sensitivity of conventional methods, determine the minimum bactericidal concentration [3, 7].

**Table 3-1 The criteria of interpretation of the sensitivity of bacteria**

| **Category sensitivity of a microorganism** | **Microbiological characteristics** | **Clinical characteristics** |
|---|---|---|
| **Sensitive** | Has not mechanisms of resistance | The successful treatment with conventional doses |
| **With intermediate resistance** | Subpopulation, located between the sensitive and resistant | Therapy is successful when using maximum doses or when the infection in areas where antibiotic accumulates in high concentrations |
| **Resistant** | Has the mechanisms of resistance there is | No effect of therapy with use maximum doses |

**The minimum bactericidal concentration (MBC)** - the lowest concentration of antibiotic (mg/l or µg/ml), which in the study of *in vitro* causes the death of 99.9% of microorganisms from the initial level over a certain period of time.

The minimum bactericidal concentration (MBC) is the lowest concentration of antibiotic (mg/l or µg/ml), which in the study of *in vitro* causes the death of 99.9% of microorganisms from the initial level over a certain period of time.

The value of the MIC is used during therapy with antibiotics, has bacteriostatic, or in the absence of effect from antibacterial therapy in special patients. Particular cases to determine the MBC can be, for example, bacterial endocarditis, osteomyelitis or generalised infection in patients with immunodeficiency States.

In conclusion, I would like to note that to date there are no methods that would with absolute certainty to predict the clinical effect of antibiotics in treatment of infectious diseases. However, data of results of susceptibility can serve as a good guide clinicians to select and correction of antibacterial therapy [1, 5].

**4. Illustrative material:** a media projector.

**5. Literature:**

1. Medical Microbiology, Virology and Immunology. Part 1. General Microbiology & Medical Immunology – Lecture Course for students of medical universities / I.I. Generalov. – Vitebsk, - VSMU. - 2016. - 282 p.

2. Subhash Chandra Parija - Textbook of Microbiology and Immunology - 2nd Edition, Published by Elsevier, a division of Reed Elsevier India Private Limited: Elsevier, 2012.

3. Patrick R. Murray, PhD - Basic Medical Microbiology: textbook for universities.

4. Rajesh Bhatia, Rattan Lal Ichhpujani - Essentials of Medical Microbiology: textbook for universities. – 4th edition, Jaypee Brothers Medical Publishers *Editorial Consultant:* Ms. Peromila MA /English, 2008

5. Borisov L. B. Medical Microbiology, Virology, immunology: Textbook. M.: 000 "Medical information Agency", 2001.

**6. Checklist:**

1. Define the term "Antibiotic".
2. What is meant by chemioterapia?
3. What are common signs of antimicrobials.
4. List the spectrum of activity of chemotherapy drugs.
5. Classification of antibiotics.
6. Principles of rational antibiotic therapy.
7. What is empirical antibiotics?
8. What is etiotropic antibiotics?
9. What are the methods of determining sensitivity to antibiotics.

## LECTURE № 4.

## 1. TOPIC: INFECTION AND FORMS OF INFECTION. PATHOGENICITY OF MICROORGANISMS

**2. Purpose:** To familiarize students with the forms, periods and general concepts of the etiology and epidemiology of infectious diseases. To introduce students to the factors of pathogenicity and virulence of microorganisms and the method of biological research, widely used in the diagnosis of infectious diseases and scientific experiments.

**3. Abstracts of the lecture**

**Infection** (or ***infectious process***) is the complex of pathological process that develop as a result of multiple relationship between the virulent bacteria and the susceptible host, followed by tissue damage, organ dysfunction and subsequent stimulation of the immune response and other adaptive reactions.

Three main ***conditions*** required for the emergence of an infectious process.

The first is the presence of a virulent **pathogen**; the second is the **ability of the pathogen** to penetrate and invade the body; and the third is the **host's susceptibility** to a particular pathogen.

The intensity of the infectious process is promoted by the three conditions listed above. The first condition is based on the virulence and its dose; the second depends on the effectiveness of tissue protective barriers, and the third depends on the activity of the immune and other adaptive system [1].

According to their capacity to cause infectious process all of microorganisms are separated into three main groups: ***obligate pathogenic***, ***facultatively pathogenic*** and ***non-pathogenic*** or ***saprophytic microorganisms***.

**Obligate pathogenic microorganisms** have highly aggressive virulence factors and in most instance cause infectious diseases as the result of the initial susceptibility of most human hosts (plague yersiniae, anthrax pathogens, tetanus and clostridia botulism, etc.)

**Facultatively pathogenic microorganisms** can cause infectious diseases in conditions of insufficient host protection, for example, in patients with *immunocompromised* (***opportunistic pathogens***), and when inoculated in high doses (staphylo- and streptococci, pseudomonads, klebsiellae and many other enteric bacteria, various fungi, etc.)

**Non-pathogenic** or **saprophytic microbes** usually don't cause diseases. Many of them are the normal habitant of the human body. They can play the role of "accidental" pathogens for humans, since they only rarely cause some kind of infectious process only occasionally (i.e., with a very low or insignificant probability) [1].

Based on their relationship to their hosts, microorganisms can be classified into: ***SAPROPHYTES*** (Sapros decayed, phiton plant), and ***PARASITES.***

**Saprophytes** are free-living microbes. They are found in soil and water and play an important role in the decomposition of organic materials in nature. Saprophytes feed on dead organic matter.

**Parasites** are microbes that can establish themselves and multiply in hosts, derive nutrients from a living host. Parasitic microbes may be either *pathogens (*Greek pathos suffering; and gene produce, that is, disease-producing) or*commensals (*Latin – com with; and mensa – living together).

**Commensals** live in complete harmony with the host without causing any damage to it. The normal bacterial flora of the body consists largely of commensals. Many commensals behave as facultative pathogens in that they can produce disease when thehost resistance is lowered.

**Pathogens** are microorganisms that can cause disease in the host. They are divided into types: opportunists and primary pathogens.

**Opportunistic** pathogens rarely cause disease in individuals with intact immunological and anatomical defenses. In immunocompromised hosts these bacteria are able to cause disease. For example, E. coli is normally carried in human intestine. If they enter into urinary tract they lead to urinary tract

infection [1, 2].

**Primary pathogens** are organisms which are capable of causing disease in previously healthy individuals with intact immunity.

For the development of infectious processes it is necessary presence of three sections: *pathogenic microorganism*, *susceptible microorganism (host)* and *corresponding environment*.

For the development of infectious processes very important the ***infectious dose*** – the minimum quantity of microbe's cells which can cause infectious process. The infectious dose varies greatly among the pathogenic bacteria and depends on the type of bacteria, virulence, etc. (E.g., the infectious dose for S. typhi is $10^5$; for the V. cholerae - $10^{11)}$.

Pathogenic microorganisms can enter the human body and other hosts through several paths (routes), which called ***entry portals.*** Many pathogenic microorganisms have a preferred route of entry, which is a prerequisite for their ability to cause disease. If they gain access to the body through another portal, the disease may not occur. For example, typhoid bacteria (Salmonella typhi) cause all the signs and symptoms of the disease when swallowed (preferred route), but if the same bacteria are rubbed into the skin, no reaction occurs, or streptococci that are inhaled (preferred route) can cause pneumonia; those that are swallowed usually do not cause any signs or symptoms. Some pathogens, such as Yersinia pestis (plague), staphylococci, microorganisms can trigger the disease from more than one portal of penetration.

The most important portals of pathogen penetration are the mucous membranes, skin, respiratory tract, gastrointestinal tract, genital tract [2].

The words "virulence" and "virulent" derived from the Latin word *virulentus*, meaning "full of poison." The term *virulentus* derived from the Latin words *virus* (poison) and *lentus* (fullness), and, in turn, the term *virus* may be related to the Sanskrit word *visham*, meaning "poison."

***Virulence*** is a measure of a microbe's ability to cause disease. This is a

quantitative indicator of pathogenicity, which measured by the number of organisms needed to cause the disease. This means that a highly virulent microbe requires fewer organisms to cause disease than a less virulent one; therefore, it directly depends on the infectious dose of the organism.

The 50% lethal dose (LD50) is the number of organisms needed to kill half of the hosts, whereas the 50% infectious dose (ID50) is the number of microbes needed to infect half of the hosts. The infectious dose of the body required to cause the disease varies depending on the pathogenic bacteria. For example, the infectious dose of *Shigella* that causes dysentery is less than 100 organisms, while the dose of *Salmonella* that causes diarrhea is more than 100,000 organisms.

The virulence of a microbe is definitely by virulence factors such as capsules, exotoxins or endotoxins (Table 4-1).

**Table 4-1 Important bacterial surface virulence**

| Virulence factors Bacteria | Virulence factors Bacteria |
|---|---|
| Capsule<br>**Polysaccharide capsule**<br><br><br><br><br>**Polypeptide capsule** | *Streptococcus pneumoniae*<br>*Klebsiella pneumoniae*<br>*Haemophilus influenzae*<br>*Salmonella typhi*<br>*Neisseria meningitidis*<br>*Bacillus anthracis* |
| **Pili protein** | *Escherichia coli* |
| **Protein A** | *Staphylococcus aureus* |
| **M protein** | *Streptococcus pyogenes* |
| **V and W proteins** | *Yersinia pestis* |

***Pathogenicity*** is the capacity of a pathogen species to cause disease, while virulence is used to describe the sum of disease causing properties of a population (*strain*) within the species. Pathogens can be distinguished from their avirulent counterparts by the presence of specific genes or gene clusters in the genome known as ***pathogenicity islands***.

***Infectiousness*** (or infectivity) refers to the capability of pathogens to enter the body and cause disease, as well as the capability of microbes to be transmitted

with one of the transmission mechanisms, while maintaining their pathogenic properties in this phase and overcoming surface barriers (skin and mucous membranes). This caused by the presence of pathogenic factors that promote to its attachment to the cells of the body and their colonization.

***Under the invasiveness*** *we mean the capacity of pathogenic microorganisms to overcome the protective mechanisms of the body, multiply, penetrate into its cells and spread in it.*

***The toxicity*** of bacteria is conditioning to the production of exotoxins. ***Toxicity*** is caused by the presence of endotoxins. Exotoxins and endotoxins have a peculiar effect and cause deep disturbances in the vital activity of the organism. Infectious, invasive (aggressive) and toxigenic (toxic) properties are relatively unrelated to each other, they are manifested differently in different microorganisms [2, 5].

**Exotoxins** produced within some bacteria during their growth and metabolism and released into the environment. There are 80 bacterial exotoxins, identification of these toxins by mass, chemical structure, target cells and biological activity. Exotoxins are proteins. They consist of two different polypeptides, designated as A (active) and B (binding). Although only part A causes symptoms in the host, while part be binds to surface receptors on the host cell and causes the transport of the entire protein through the plasma membrane into the cell. Most bacteria produced exotoxins are gram-positive. Since exotoxins are soluble in body fluids, they can easily diffuse into the blood and quickly transported throughout the body. Exotoxins act by destroying certain parts of host cells or by suppressing certain metabolic functions.

**The main properties of exotoxins:**

1. Exotoxins are proteins and they are thermolabile toxins.
2. Specific action
3. They possess high antigenic and immunogenic properties
4. Since exotoxins are soluble in body fluids, they can easily diffuse into

the blood and quickly transported throughout the body.

5. Most exotoxin-producing bacteria are gram-positive.

6. Exotoxins used for preparation of anatoxins.

Exotoxins may grouped into following principal types, based on their mode of action:

***1.*** **Cytotoxins** – which kill host cells or affect their functions. They are inhibits protein synthesis in Eukaryotic cells (Diphtheria toxin).

*a) Antielongaters* – they suppress protein synthesis in Eukaryotic cells: antielongaters, which suppress transferase II enzyme (e.g., Diphtheria histotoxin).

*b) Enterotoxins (S. aureus, C. perfringens)*

*c) Dermonecrotoxins* (*S. pyogenes*, *B. pertussis*, *B. anthracis*, *P. aeruginosa*)

***2.*** **Membrane toxins** – hemolysins and leukocydins. These toxins raise the permeability of membranes.

***3.*** **Functional blockaters** – which are divided into several groups:

*a) Enterotoxins (thermostable and thermolabile)* – thermolabile enterotoxin induces the formation of cyclic AMP from ATP in the cytoplasm. As a result, epithelial cells secrete a large amount of fluid and electrolytes (ions). Normal muscle contractions are disrupted, which leads to severe diarrhea, which can be followed by vomiting (Cholera toxin). Thermostable enterotoxin activates guanilate cyclase (Y. enterocilitica)

*b) Neurotoxins* - Hinder the transmission of impulses from the nerve cell (Botulinum toxin-blocks transmission of nerve signals to the muscles by preventing the release of acetylcholine; Tetanus toxin-blocks the action of inhibitory neurons by preventing the release of neurotransmitters).

*c) Toxicoblokaters* – they render the opposite action of the enterotoxins. These toxins cause accumulation of cAMP in tissues and development of oedema (plague, anthrax).

***4.*** **Erythrogenic toxin and Exfoliatin** toxin. *S. pyogenes* produces erythrogenic toxins that damage the blood capillaries under the skin and cause a

red skin rash. S. aureus produces exfoliatin toxin which damage intracellular bonds and cause impetigo in the new–born.

The body create antibodies called antitoxins that provide immunity to exotoxins (antitoxic immunity). When exotoxins deactivated by heat and formaldehyde, or other chemicals, they no longer cause disease, but are still able to stimulate the production of antitoxin, so that immunity to the disease is developed. Diphtheria and tetanus can be prevented by toxoid vaccination (anatoxins).

Protein toxins are classified into three classes:

1.Class A - fully secretion of the toxin into environment (histotoxin)

2.Class B - partial secretion of this toxin (tetanospasmin)

3.Class C – (unrealizable) liberation of this toxin can occur after lysis of bacteria(plague's mice toxin) [5].

**Endotoxins** vary from exototoxins in several ways. Endotoxins are ingredient of the outer part of the cell wall of gram-negative bacteria. Gram-negative bacteria have an outer membrane surrounding the peptidoglycan layer of the cell wall. This external membrane consists of lipoproteins, phospholipids, and lipopolysaccharides (LPS). The lipid part of the LPS, called **lipid A,** is an endotoxin. Thus, endotoxins are lipopolysaccharids, whereas exotoxins are proteins.

Endotoxins have their effect when gram-negative bacteria die and their cell wall undergoes lysis, thus releasing endotoxin. Antibiotics used to treat diseases caused by gram-negative bacteria can lyse bacterial cells; this reactions releases endotoxin and can lead to instant worsening of the symptoms, but the condition usually increase as the endotoxin break down. All endotoxins cause the same signs and symptoms, regardless of the type of microorganism, although not to the same extent. Host reactions include chills, fever, weakness, general pain, and in some cases shock and even death. Endotoxins can also cause miscarriage and prevent blood clotting.

The shock caused by gram-negative bacteria is called **septic (**or endotoxic**) shock.**

Endotoxins do not contribute to the formation of effective antitoxins. Endotoxins are weakly antigenic; they stimulate protective antibodies so poorly. Antibodies are produced, but they usually do not counteract the action of the toxin; sometimes, in fact, they actually enhance its effect. No anatoxins were obtained from endotoxins. Endotoxins are not used as antigens in any available vaccine. Typical microorganisms producing endotoxins are Salmonella typhi (the causative agent of typhoid fever), Neisseria meningitides (the causative agents of meningococcal meningitis).

The factors of virulence are:

**Adherence** – (attachment) is an important step in pathogenicity Attachment between pathogen and host is performed by surface molecules on the pathogen called adhesions or ligands that specifically bind to additional surface receptors on cells of certain host tissues. The adhesives can be located on the glycocalyx of the microbe or on other microbial surface structures, such as pili (fimbriae). Most adhesives on microorganisms are glycoproteins, lipoproteins, lipoteichoic acid, fimbriae and capsule. The receptors on the host cells are usually sugars, such as mannose. Adhesives on various strains of the same type of pathogen may differ in structure. Different cells of the same host may also have different receptors, which differ in structure. If the adhesins, receptors, or both can be altered to interfere with adhesion, infection can often be prevented (or at least controlled). Receptors of the host are following: **native, inductive and acquired.**

***1. native*** – located on epithelial cells and participates in the adhesion of specific bacteria

***2. the inductive*** effect can manifest itself only after the absorption of viruses by susceptible cells

***3. acquired*** – appears under special conditions. These receptors are bridge crossing between epithelial cells and bacteria. The role of bridge crossing is played by immunoglobulin, a component of the complement system.

**Colonization** is the up growth and reproduction of bacteria.

**Invasion and penetration: Invasion** when the microorganism penetrates through mucous and connective barrier (Staphylococcus, Streptococcus). Several enzymes allocated by invasive bacteria play the role in pathogenesis. Among the most famous are: hyaluronidase, neuraminidase, collagenase, which destroy collagen and hyaluronic acid, respectively, thereby allowing bacteria to spread through subcutaneous tissue. **Penetration** – when the microorganism penetrates into epithelial cells, leukocytes and lymphocytes(Shigella, *E. coli*).

**Aggression** – several virulence factors promote to invasiveness by limiting the ability of the host defense mechanisms, especially phagocytes, to operate effectively.

The most important of these antiphagocytic factors is the capsule outer to the cell wall of several important pathogens, such as S. pneumoniae and Neisseria meningitidis.

The second group of antiphagocytic factors are proteins of the cell wall of the gram-positive cocci, such as **M protein** of S. pyogenes and **protein A** of S. aureus. The M and A proteins are antiphagocytic, they bind to the Fc fragment of IgG and warn the complement activation and inhibit phagocytosis.

The third group factors, which repress the host defense are **enzymes**:

a) protease, which collapse IgA, permitting the organism to adhere to mucous membranes and is produced chiefly by *N. Gonorrhea, S. pneumonia* and etc.

b) coagulase, which is prepared by S. aureus and speed up the formation of a fibrin clot from its precursor, fibrinogen (this clot may protect the bacteria from phagocytosis by walling off the infected area and by coating the organisms with a layer of fibrin).

c) leucocidins, which can destroy both neutrophilic leukocytes and macrophages.

The diseases that can be spread from one person to another are called communicable diseases. Most microbial infections are communicable diseases [3].

Three epidemiological terms are often used to describe infection: endemic, epidemic, and pandemic:

■ **Endemic:** The infection that occurs at a persistent, usually low level in a certain geographical area is called endemic.

■ **Epidemic:** The infection that occurs at a much higher rate than usual is known as epidemic.

■ **Pandemic:** Infection that spreads rapidly over large areas of the world is known as a pandemic [3].

In nature infectious diseases are subdivided into *exogenous* and *endogenous*.

E**xogenous infections** – the causative agent penetrates into the macro-organism from the environment (from patients, carriers, from foodstuffs, water, air, soil and etc.).

**Endogenous diseases (auto - infections**) originate as a result of the activation of the indigenous microbes of the body due to disturbances of the internal medium of the macro-organism as a result of external factors and social conditions. The state of autoinfection is quite a wide spread phenomenon.

In some cases infection causes a weakening of the body which then becomes susceptible to other diseases. Thus e.g., after influenza or measles pneumonia occurs. This is known as *secondary infection*. There are also ***local*** and ***generalized*** infections. For example, during infection with staphylococcus, the infectious process causes furunculous (local infection), and if the causative agent penetrates into the blood sepsis will develop (generalized infection).

**Types of infections.** Infections may be of the following types:

**Primary infection:** This condition denotes an initial infection with an organism in a host.

**Reinfection:** This condition denotes subsequent infection with the same organism in the same host.

**Secondary infection:** This condition denotes an infection with a new organism in a host whose body resistance is already lowered by a pre-existing infectious disease.

**Cross-infection:** This condition denotes an infection with a new organism from another host or another external source in a patient who is already suffering from a disease.

**Nosocomial infection:** Cross-infections acquired in hospitals are called hospital-acquired, hospital-associated, or nosocomial infections.

**Iatrogenic infection:** This condition denotes a physician induced infection as a result of therapy with drugs or investigation procedures.

**Subclinical infection:** Inapparent clinical infections are called subclinical infections.

**Latent infections:** This denotes a condition in which some organisms may remain in a latent or hidden stage in host and subsequently they multiply to produce clinical disease when host resistance is lowered.

**Stages of Pathogenesis of Infections**

Infectious diseases are complex. The outcome of infection depends on a variety of factors of the microbe and host as follows:

**1.** The ability of the organism to break host barriers and to evade destruction by innate local and tissue host defenses.

**2.** The ability of the organism to replicate, to spread, to establish infection, and to cause disease.

**3.** The ability of the organism to transmit to a new susceptible host.

**4.** The innate and adaptive immunologic ability of the host to control and eliminate the invading microorganism.

The infection process involves the following stages: (*a*) transmission of infection, (*b*) entry of the organisms and evasion of the local defenses, (*c*) adherence to cell surfaces, (*d*) growth and multiplication of the bacteria at the site of sticking, (*e*) manifestations of disease, and ( *f* ) termination of disease [3].

**Dynamics of infectious diseases**

To preserve an infectious disease, there must be a reservoir of microorganism from which the pathogen must transmitted to a susceptible host either straight or through the medium of a vehicle or a vector (Figure 4.1).

***Source and Reservoir.*** The source of infection is the person, animal, object or substance from which an infectious agent is transmitted or spread to the host, whereas a reservoir is defined as any person, animal, arthropod, plant, soil or substance (or combination of these) in which an infectious agent lives and reproduces. These can be of three types: Humans, animals and non-living substances.

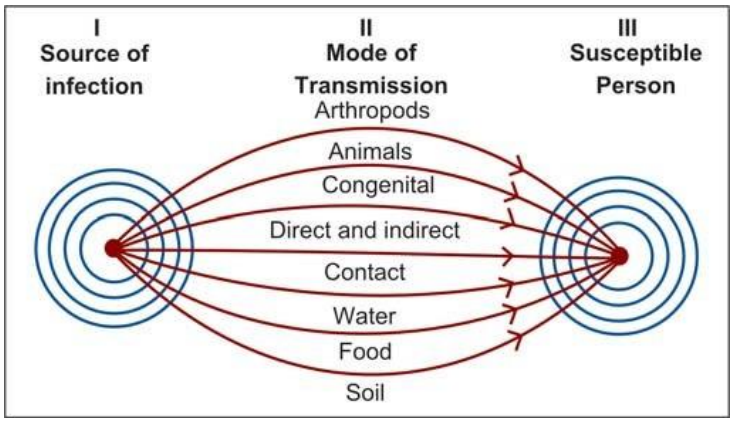


**Figure 4.1.** Sources and vehicles of infections

**Humans**. For infectious diseases of human beings, humans are the most important reservoir. It can be a *case or a carrier*.

**Human Case.** The patient may have a clinical disease or a subclinical infection that remains undiagnosed or abortive. In the latter case, the causative agent of the disease can multiply in the host body, but does not manifest itself with signs and symptoms.

**Human Carriers.** Some microorganisms are not completely eliminated from the host body after the natural cycle of the disease or after cure. Such persons make carriers of the agents. A carrier is defined as an infected person or animal that carries a specific infectious agent in the absence of obvious clinical signs and serves as a potential source of infection for others. Although carriers are less contagious than patients, they are of great epidemiological importance due to the long period during which they can secrete organisms unnoticed.

The carriers can be: *incubatory, convalescent or healthy*.

According upon the period of allocation of microorganisms they can be designated as: *temporary (acute) or chronic carriers.*

The incubatory and recovering carriers are usually temporary whereas chronic carriers are otherwise healthy individuals. Chronic carrier state occurs in various diseases especially typhoid fever and hepatitis B.

***Animals.*** Animals and birds can also transmit microorganisms to humans. They can also manifest as a case or exist as carriers. Diseases that are naturally transmitted between humans and animals are called ***zoonoses.*** These diseases are of great importance in countries where close contact between humans and animals is inevitable. Some of the important zoonotic infections are rabies, plague, brucellosis, leptospirosis, hydatidosis.

***Non-living Substances.*** Soil and inanimate objects can also be reservoirs of some microorganisms, such as pathogens of tetanus, anthrax, hookworms and mycetomas [4].

**Modes of Transmission.** Microorganisms can be transmitted to humans directly or indirectly. Direct transmission takes place via: contact with human, animal or inanimate objects; drip infection; damage to the skin or mucous membrane and transplacental and congenital.

*Indirect transmission* is possible using any of the following mechanisms:

- Vehicles (water, food, etc.)
- Carriers (mechanical or biological)

- Air (drops or dust)
- Fomites

Unclean hands and fingers.

**The mechanism of infection**. To cause infection in humans, the microbe must enter the host's body. The most frequent routes of penetration are the respiratory tract, gastrointestinal tract and ruptures of the superficial mucous membranes and skin. From the portal of penetration, the parasite can spread directly through the tissues or can pass through the lymphatic channels into the bloodstream, which distributes it widely and allows it to reach tissues especially suitable for its reproduction. Nevertheless, for the preservation of the parasitic species, a satisfactory exit portal of the parasite from the host and an effective mechanism of transmission to new hosts are also of important importance (Figure 4.2).

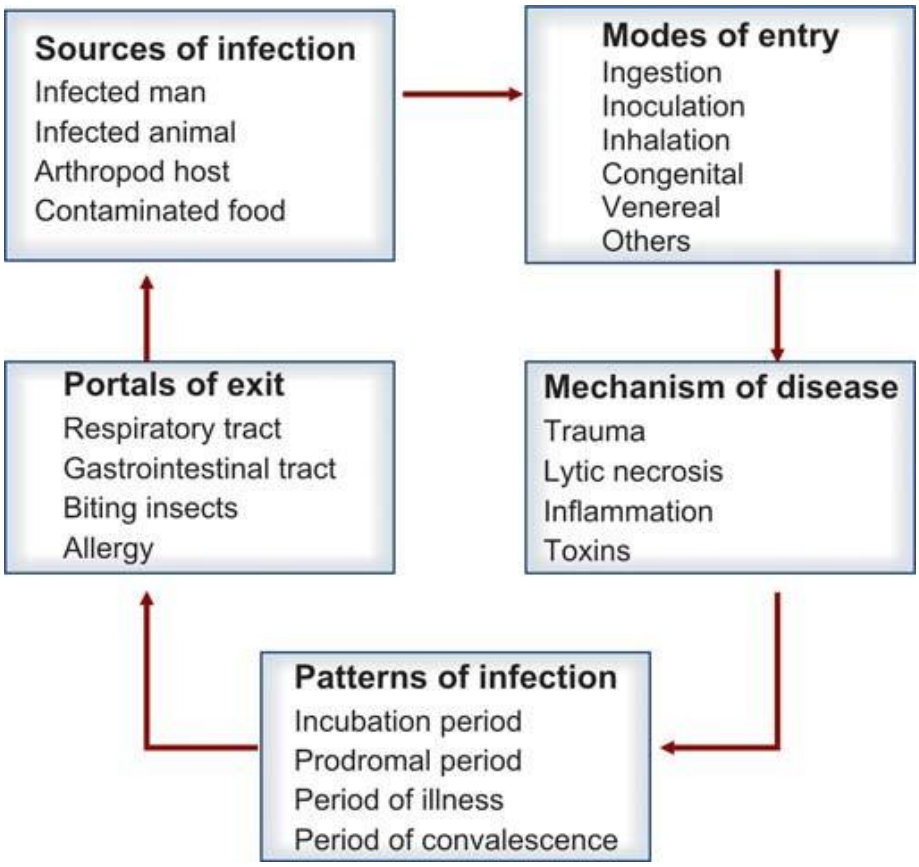


**Figure –4.2.** Dynamics of infection

**Stage of infectious process.**

**The incubation period** — [from the Latin. *incubatio* "hatching Chicks"]. Usually between the penetration of infectious agent into the body and the manifestation of clinical signs there is a specific for every disease the period of time of the incubation period, is characteristic only for exogenous infections. During this period, the pathogen multiplies, there is an accumulation as a causative agent and provide them with toxins to a certain threshold beyond which the body begins to respond clinically severe reactions. The incubation period can vary from hours and days to several years [4].

**Prodromal period**. Usually, the initial clinical manifestations there are no pathognomonic for a specific infection characteristics. The usual weakness, headache, feeling of weakness. This stage of infectious disease is called the prodromal period, or "phase precursors". Its duration does not exceed 24-48 h [3].

**The period of development of the disease** — during this phase, the traits of individuality of the disease or common to many infectious processes signs (fever, inflammatory changes etc.). In the symptomatic phase it is possible to identify stages of onset of symptoms (stadium incrementum), the heyday of the disease (stadium acme) and decline of the manifestations (stadium decrementum). [3]

**Recovery** — [from lat. *re-*, repeated actions, + *convalescentia*, recovery]. The period of recovery, or convalescence, as the final period of infectious disease can be rapid (crisis) or slow (lysis) and also characterized by the transition to a chronic condition. In favourable cases, the clinical manifestations usually disappear faster than there comes normalization of morphological violations of organs and tissues and the complete removal of the causative agent from the body. Recovery may be full or be accompanied by the development of complications (eg, CNS, musculoskeletal or cardiovascular system). The period of the final removal of the infectious agent may be delayed for some infections (eg, typhus) could amount to decades. [3]

**Table 4-3 Microbiological and immunological characteristic of periods of infectious diseases**

| Period of infectious disease | Behavior of causative agent | Discharges of causativeagent into environment | Immune responses |
|---|---|---|---|
| **1. Incubation** | Adhesion on the specific receptors of susceptible cells. | As a rule discharges isnot detected. | Antibodies are not detected. |
| **2. Prodrome period** | Colonization of the susceptible cells. Manifestation of the first non-specific symptoms. | The same. | The same. |
| **3. The specific illness period** | Intensive reproduction of causative agent. Manifestation of specific symptoms. | Discharged. | Appearance of immunoglobulins class M, at the end of the period changes IgM into IgG and IgA is occurring. |
| **4. Convalescence (recovery)** | Cessation (stopping) of the reproduction and death of causative agent. Normalization of functions of the patient. | Stopping of the discharging of the causative agent or carrier state can occur. | Increasing of the titer of IgG, IgA. In some cases, immediate type of hyper-sensitivity can develop. |

**4. Illustrative material:** tables, charts, posters, multimedia projector.

**5. Literature:**

1. Medical Microbiology, Virology and Immunology. Part I. General Microbiology & Medical Immunology – Lecture Course for students of medical universities / I.I. Generalov. – Vitebsk, - VSMU. - 2016. - 282 p.

2. Borisov L.B. Medical Microbiology, Virology, Immunology. -M .: MIA, 2006.-734 p.

3. Subhash Chandra Parija - Textbook of Microbiology and Immunology - 2nd Edition, Published by Elsevier, a division of Reed Elsevier India Private Limited: Elsevier, 2012.

4. Rajesh Bhatia, Rattan Lal Ichhpujani - Essentials of Medical Microbiology: textbook for universities. – 4th edition, Jaypee Brothers Medical Publishers *Editorial Consultant:* Ms Peromila MA (English), 2008

5. Medical Microbiology, Virology and Immunology. Ed. Vorobeva A.A. - M .: MIA, 2008. - 690 p.

**6. Checklist:**

1. Factors of pathogenicity of bacteria.
2. Methods of obtaining toxoids, their practical significance and application.
3. Forms of infection and their characteristics.
4. Periods of infectious disease.
5. Sources of infection.
6. Stage of infectious process.
7. Modes of Transmission.
8. The reservoir of infection.
9. Stages of Pathogenesis of Infections.
10. How many factors of virulence?

## LECTURE № 5.

## 1. TOPIC: IMMUNOLOGY. THE DOCTRINE OF IMMUNITY. BASIC PRINCIPLES OF THE ORGANIZATION AND FUNCTIONING OF THE IMMUNE SYSTEM

**2. Purpose:** Explain to students the non-specific factors of immunity, types of immunity, the essence of the immunology section. Analysis of the stage of phagocytosis. Explain the classification of complement.

**3. Abstracts of the lecture**

### HISTORICAL BACKGROUND OF IMMUNOLOGY

Immunology defined as the study of the molecules, cells, organs, and systems responsible for the recognition and removal of foreign material. Immunology emerged as a branch of microbiology. The study of infectious diseases and the body's response to them played an important role in the development of immunology. In addition, the concept of the microbial theory of disease contributed to the development of immunology. Edward Jenner first studied the body's response to foreign substances. He observed that dairymaids who naturally contracted a mild infection called cowpox were protecte from smallpox, a horribly disfiguring disease and the ultimate killer.

In 1796, Jenner vaccinated an eight-year-old boy with liquid from blisters of cowpox on the hand of a milkmaid. The boy got sick with cowpox. Jenner then vaccinated him two months later with smallpox blister fluid, but the boy only evolved a small sore at the inoculation site. Exposure to a mild form of cowpox made him immune to smallpox. These were some of the most important developments in the history of immunology since Jenner's achievement. In 1879 Neisser isolated the first human pathogen-gonococcus. In 1883 Klebs and Loeffler isolated diphtheria bacilli, leading to the isolation of the first definite antigen,

diphtheria toxin, by Roux and Ersin in 1888. In the same year, Nuttall and Pasteur was informe about the first antibodies, serum bactericidins. In 1890, von Behring and Kitasato founded antitoxins, which led to the development of toxoids for diphtheria and tetanus. In 1900 Land Steiner founded blood group antigens and their corresponding antibodies. This led to the possibility of blood transfusions without provoking reactions. It was in 1916 that the first journal of immunology began published, in which many new discoveries were publishe. In general, immunology has always depended on and stimulated the use of technology, such as the use of microscopy, electrophoresis, immune electrofluorescence, etc. Thus, immunology did not become an innate discipline, but maintained close ties with many other areas of the medical sciences [1, 2, 3].

**Immunology** is the study of immunity. Immunity is a complex of physiological defence reactions which determine the relative constancy of the internal medium of the microorganism, hinder the development of the infectious process or intoxication, and are capable of restoring the impaired functionsof the organism.

**Immunity** is the main defense mechanism for humans. Recovery from illness is the result of his struggle with disease agents. It is interesting that infectious diseases, such as measles or rubella, happen to people once in their lives. Because, having transferred them once, the body produces immunity, and it is no longer susceptible to this kind of infections [3].

Modern classification subdivides immunity into two types according resistance:

1. **Natural** - congenital, hereditary, nonspecific immunity or nonspecific resistance. Acts on antigens nonspecifically, is the primary inflammatory protective reaction to the antigen. It was formed in ontogenesis.

2. **Acquired** - adaptive, specific. They formed when the cells of the immune system interact specifically with an antigen, resulting in antibodies that specifically recognize that antigen. They formed throughout life.

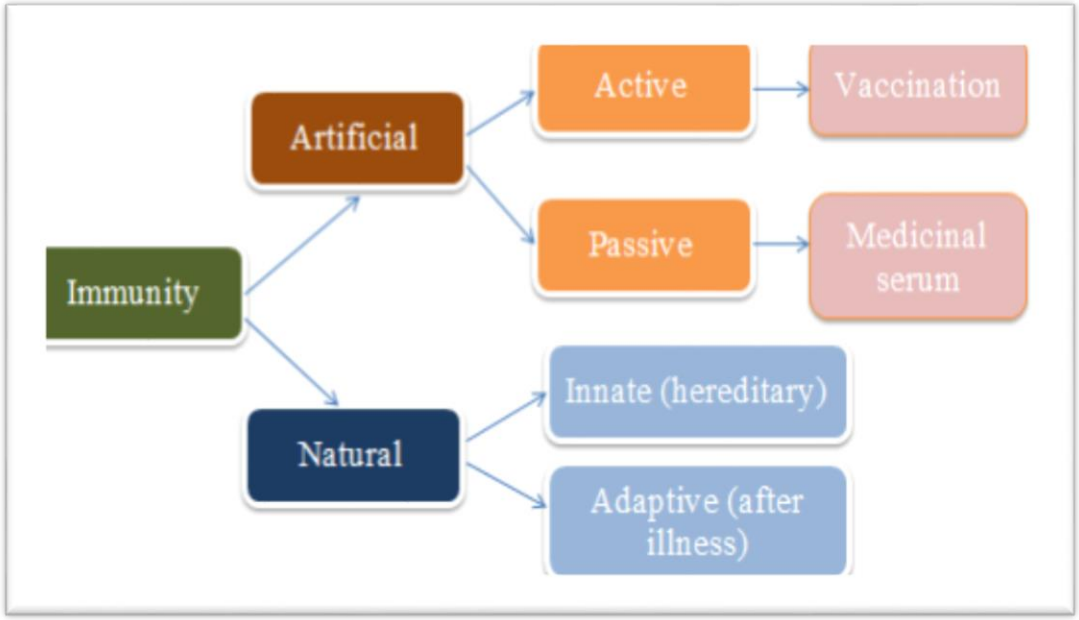


**Figure 5.1 Types of immunity**

It is natural and acquired, active and passive.

***Natural active immunity*** arises as a result, of contact with the pathogen (after having had the disease or after latent contact without symptoms of the disease - post-infection). It persists throughout life.

***Natural passive immunity*** results from the transmission from the mother to the fetus through the placenta (transplacental) or breast milk of Ig G - maternal antibodies and ready protective factors - lymphocytes, antibodies, cytokines. It lasts about 6 months.

***Acquired active immunity*** occurs after injection of vaccines and toxoids (postvaccines), which contain microorganisms or their substances - antigens. It lasts throughout life.

***Acquired passive immunity*** occurs after the introduction of ready-made antibodies or immune cells into the body. In particular, such antibodies were found in the serum of immunized donors or animals. It persists as the injected antibodies

are used.

***Natural immunity*** conditioned to mechanical barriers and factors that prevent the entry of infection into the body. This conditioned to various natural resistance mechanisms. Such factors refers to intact skin, secretions (tears, urine, sputum, saliva and other body fluids), as well as various epithelial cells and airway villi that prevent direct contact of the internal environment with a foreign agent.

**The immune system *(IS)*** is a collection of lymphoid organs and clusters of lymphoid cells. The total mass of lymphoid organs in humans is 1.0–2.5 kg. It is an independent system: it is generalized throughout the body, its cells recycle through the bloodstream throughout the body, and it has the ability to produce specific antibodies to the antigen. The main cells of the IS are lymphocytes. The immune system does not always provide only protective reactions aimed at maintaining health. With her active participation, autoimmune, allergic and other immunopathological reactions develop. The immune system includes central and peripheral organs (Figure 5.2.) [4].

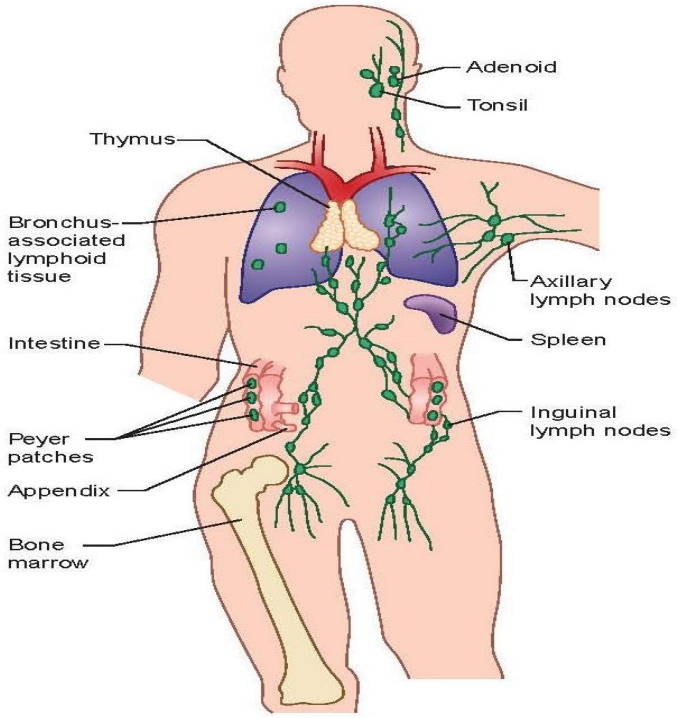


**Figure 5.2. Central and peripheral lymphoid organs and tissues**

In addition, the IC is subdivided into encapsulated organs (thymus, spleen, lymph nodes), reencapsulated lymphoid tissue of mucous membranes of gastrointestinal tract, respiratory system, lymphoid system of skin (skin cells, regional lymph nodes and lymphatic vessels) and other mucous membranes. The organs of the immune system contain afferent (blood or lymph), efferent (outflow) vessels; sinuses, areas of cell reproduction, maturation and differentiation. Fibrous tissue structure [3, 4].

***Natural Resistance Mechanisms:*** a) the protective role of the skin and mucous membranes; b) normal microflora of a microorganism; c) inflammation; d) fever; e) the barrier role of lymph nodes; f) function of the excretory system; g) humoral factors; h) phagocytosis.

***General properties of nonspecific resistance factors:***

a) are formed in the body before the introduction of foreign agents and can be activated almost immediately after the appearance of a foreign microorganism;

b) under conditions of biological aggression are activated during the development of an inflammatory reaction;

c) pathogens themselves and damaged cells of the body are the source of activation;

d) chemotactic signal emanating from the focus of inflammation promotes penetration of circulating leukocytes into the site of damage and development of local protective reactions;

e) activated leukocytes produce bactericidal substances;

f) destruction of foreign agents is carried out in the form of extracellular, intracellular and contact cytolysis;

g) contact cytolysis is mediated by apoptosis.

Under the phagocytosis understand the absorption cell particles larger than 0.5 microns. As already noted, the phenomenon of phagocytosis was opened by I. I. Mechnikov (1882). He showed the fundamental role of phagocytosis as a method to supply single-celled organisms evolved into multicellular defense mechanism against foreign agents. Currently, the role of phagocytosis in the body of a multicellular consider even wider: we have shown its involvement in morphogenesis, elimination of cells dying through apoptosis, etc.

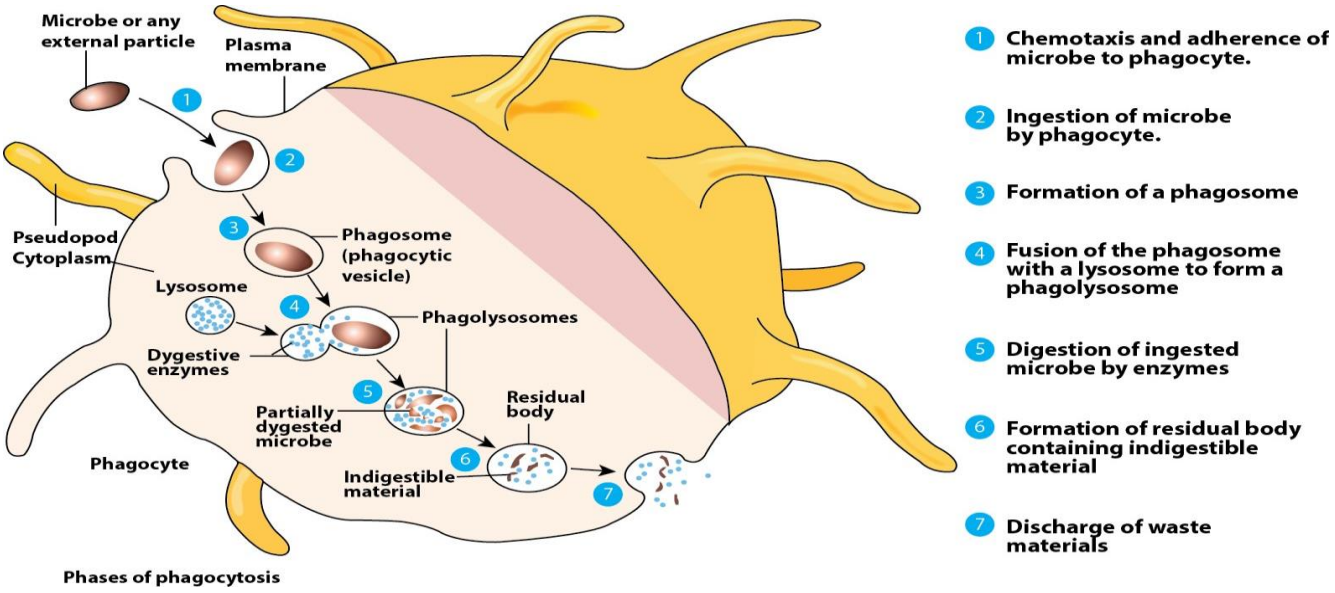


**Figure 5.3. Phases of phagocytosis**

Traditionally isolated 8 stages of phagocytosis (Figure 5.3.):

1) chemotaxis (directional movement of the phagocyte toward an object);

2) adhesion (attachment to the object);

3) activation of a part of the phagocyte membrane (actinomyosin system of the phagocyte);

4) initiation of phagocytosis itself, associated with the formation of pseudopodia around the absorbed particle;

5) phagosome formation;

6) fusion of phagosome with lysosomes;

7) destruction and digestion;

8) the release of degradation products.

Numerous cells absorb foreign material, but the ability to increase this activity in response to opsonization by antibodies and/or complement and to acquire specificity to the antigen is unique to myeloid cells, namely polymorphonuclear leukocytes, monocytes and macrophages, and they called professional phagocytes.

Chemotaxis of phagocytes discussed by us above, as it relates to the process of emigration of leukocytes from the blood stream. Chemotaxis is a necessary prerequisite for migration, regardless of the starting position of the phagocyte (blood flow, inflammation, etc.). Chemotaxis can be viewed as a distant interaction of the phagocyte and its object. Thanks prior to chemotaxis to the beginning of phagocytosis, the cell is polarized: the filaments of the cytoskeleton and organelles are oriented in the direction of the source of chemotactic signals (usually the object of phagocytosis), and membrane molecules necessary for the implementation of phagocytosis localized at the pole of the cells facing the target. Consider the phagocytosis phase of adhesion, in which cells of the phagocytic process participants interact physically [5, 6].

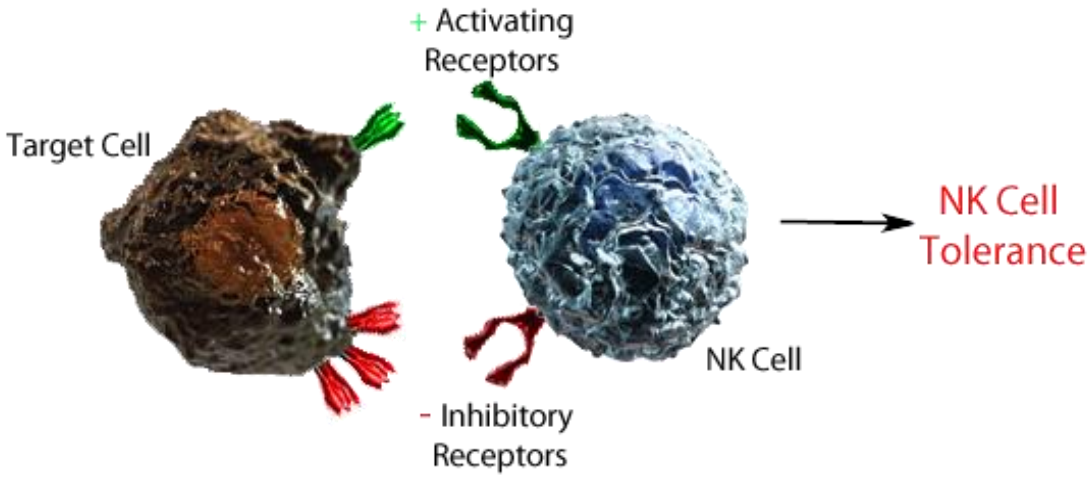


**Figure 5.4. Natural killer cell**

**Natural killer cells (NK) –** play an important role in the innate host defences. NK cells are lymphocytes (they contain 5-10% of peripheral

lymphocytes) with some T cell markers, but they do nothave to pass through the thymus in order to mature (Figure 5.4.). NK cells are capable of destroying especially virus- infected cells and tumour cells by secreting cytotoxins (perforins, granzymes). In contrast to cytotoxic T cells, they do not seem to be immunologically specific; that is, they do not need to stimulate by an antigen. They are not need phagocytic but must contact the target cell to lyse it. The functions of natural killer cells are to attack and destroy target cells. They can kill without antibody, but antibody enhances their effectiveness a process called antibody-dependent cell-mediated cytotoxicity. They have no immunologic memory and, unlike cytotoxic T cells, have no T cell receptors also killing does not require recognition of MHC proteins. NK cells have receptors that detect the presence of class IMHC proteins on the cell surface. If a cell displays sufficient class I MHC proteins that cell not killed by the NK cells. Virus infected cells and tumour cells display a significantly reduced amount of class I MHC proteins, and it is those cells that are recognized and killed by the NK cells.

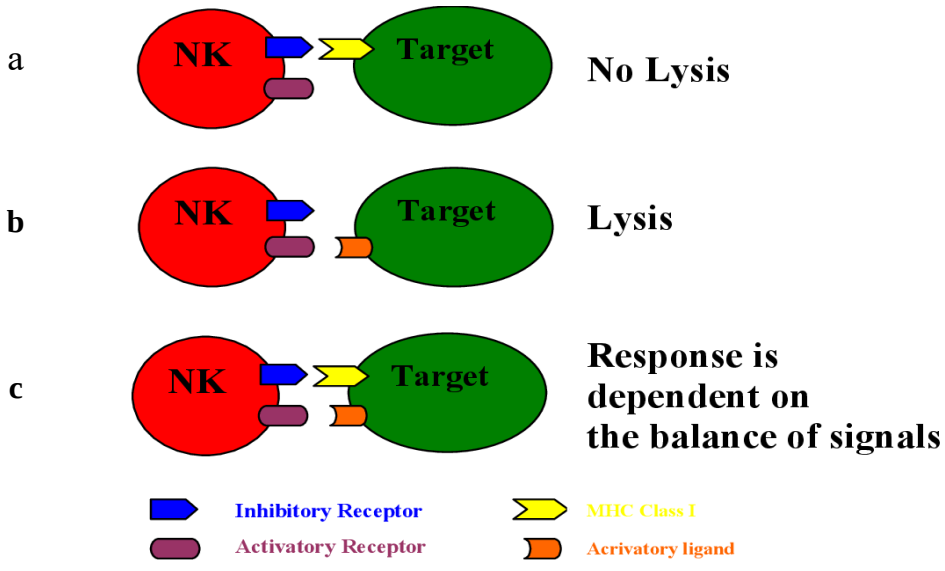


**Figure 5.5. The "missing self" hypothesis.**

The figure 5.5. shows the three types of interaction of NK-cells with targets. On NK cells there are two types of recognition receptors: activation and inhibitory. Inhibitory receptors distinguish MHC-I molecules and inhibit the signal from activation receptors, which, in turn, determine either MHC-I molecules (but with less affinity than inhibitory receptors) or MHC-like molecules:

a – target cell does not express activation ligands and lysis does not occur;

b - the target cell expresses the activation ligands, but does not express MHC-I. Such a cell undergoes lysis;

c - cells of the target contains both MHC-I molecules and activation ligands. The outcome of the interaction depends on the balance of signals coming from the activation and inhibitory receptors of NK cells [3, 5].

**Complement –** the body produces certain antimicrobial substances. Among the most important of these are the proteins of the complement system. The complement is a defense system consisting of about 20 proteins that are present in normal human (and other animal) serum, nine of which are major C1-C9 fractions. The term "complement" refers to the ability of these proteins to complement, that is, amplify the action of other components of the immune system, such as antibodies. The complement is an important component of the body's innate defense. Complement proteins synthesized mainly by the liver and mononuclear phagocytes. Proteins of the complement system make up about 5-10% of blood serum proteins.

The complement is thermolabile and inactivated by heating serum at 56º C for 30 minutes.

Activation of complement system occurs by three pathways (Figure 5.6.):

1. **Classical pathway** - activation initiated by antigen-antibody complex (**Ag+IgM; Ag+IgG** -the systemcan be activated by an immune reaction).

**2. Alternative pathway** – activation complement system can be initiated by a variety of non- immunologic molecules (endotoxin, the particles of

viruses or bacteria and etc.) and formation of antigen-antibody complexes (**Ag+IgA**; **Ag+IgE)**

3. **Lectin pathway** – activation by the lectin pathway requires mannose-binding lectins (MBLs) without participation of immunoglobulins. Blood specific proteins can bind mannose which is in the bacterial membrane. This interaction activate C4 fraction after it activation by classical pathway.

**Classical pathway -** In the classical pathway, antigen-antibody complexes activate C1 to form a protease that cleaves C2 and C4 to form the C4b2a complex. The latter is a C3-convertase that cleaves C3 molecules into two fragments, C3a and C3b. C3a is an anaphylatoxin. C3b forms a complex with C4b2a, producing a new enzyme, C5-convertase, which cleaves C5 to form C5a and C5b. C5a is an anaphylatoxin and a chemotactic factor. C5b binds to C6 and C7, forming a complex that interacts with C8 and C9 to form a **"membrane attack»** complex, **C5b - C6 - C7 - C8 - C9,** which causes cytolysis. The C5a fragment is detached and has other activities (e.g., C5a can promote acute inflammation; C5a is also a chemotactic factor that attracts phagocytes to the site of complement formation).

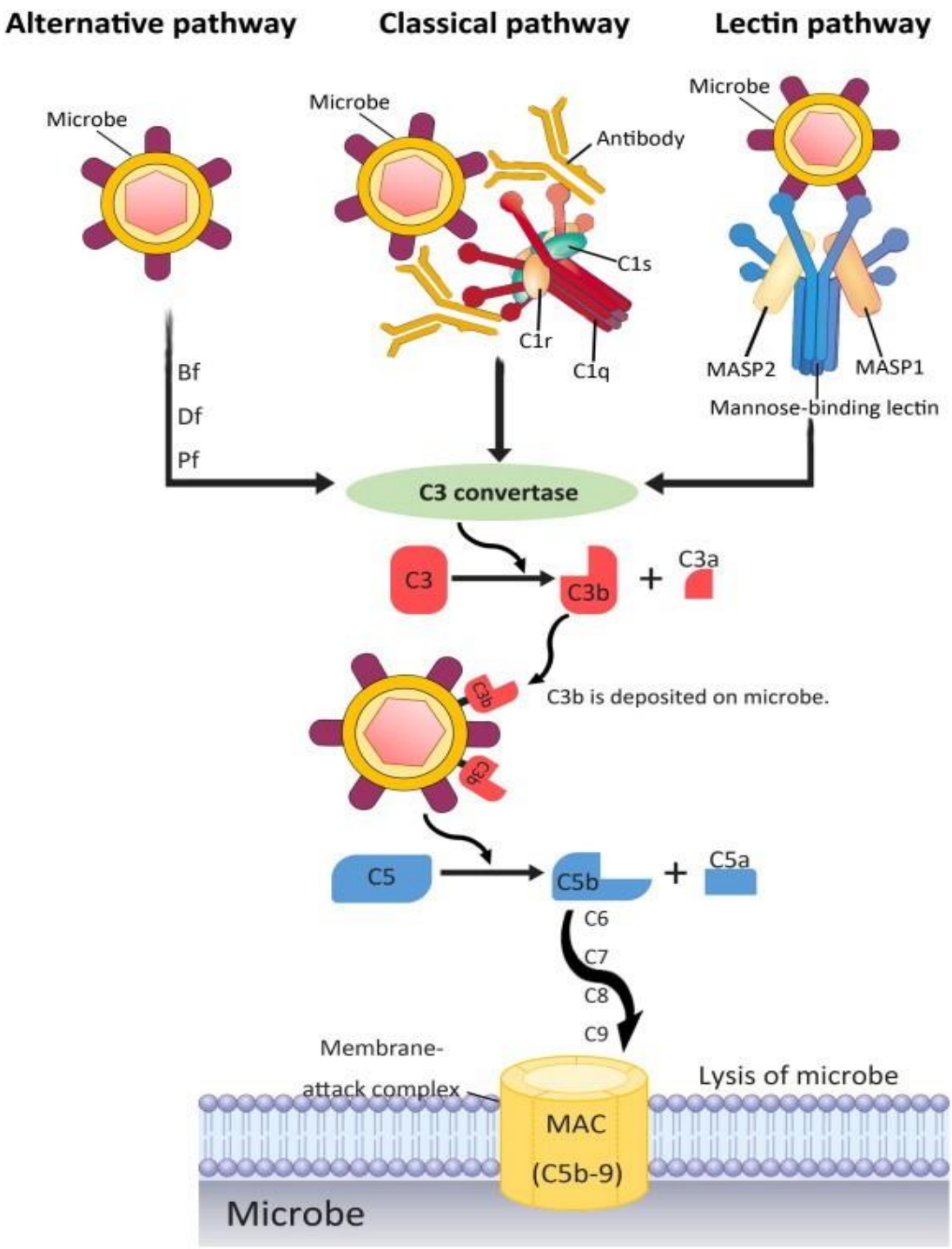


**Figure 5.6. Three pathways of activation of complement system**

**Alternative pathway –** This pathway does not require the presence of specific antibodies. A widerange of chemically unrelated substances are known to activate alternative pathway: certain polysaccharides and lipopolysaccharides, the proteins of bacterial cells superficial structures of

viruses, Immune complexes including IgA and IgE. During this processes factors B, D, P and $Mg^{2+}$ ions take place. Factor D in active form is protease which split the factor B into Bb which plays role of C3- convertase in alternative pathway. Role of P (properdin) is activation and stabilization of C3a and Bb.

Factors B, D, P react with C3 to produce C3b in the serum. The alternative pathway is initiated when C3b, factor B, factor D, and factor P combine with certain polysaccharides. After, activation of "**membrane attack**" complex **– C5b – C6 – C7 – C8 – C9** is occurs, like in classic pathway. Note thatthis pathway does not involve C1, C2 and C4.

The alternative pathway is of particular importance in combating enteric gram-negative bacteria. The outer membrane of the bacterial cell wall contains a lipopolysaccharide that is an endotoxin (lipid A), triggering the alternative pathway.

The main effects of complement:

**Cytolysis -** The introduction of the C5b and C6 to C9 complex into the cell membrane leads to the death or lysis of many cell types, including red blood cells, bacteria, and tumor cells. Cytolysis is not an enzymatic process; rather, introduction of the complex results in membrane disruption and penetration of water and electrolytes into the cell.

The utilization of the complement components in this process is called complement fixation; it forms thebasis of an important clinical laboratory test.

**Enhancement of antibody production** – C3b binding to its receptors on the surface of activated B-cells significantly enhances antibody production compared to antibody production by antigen-only activated B-cells.

**Chemotaxis** – C5a and the C567 complex attract neutrophils. They migrate particularly well under the action of C5a. C5a also increases neutrophil adhesiveness to the endothelium.

**Opsonization** – cells, antigen-antibody complexes and viruses phagocytized much better in the presence of C3b because of the presence of C3b receptors on the surface of many phagocytes. When bound to surface of a microorganism, C3b can interact with special receptors on phagocytes to promote phagocytosis. This phenomenon is called opsonization or immune adherence. In the process, C3b functions as an opsonin by coating the microorganism and promoting attachment of the phagocyte to the microbe.

**Anaphylatoxin** – C3a, C5a bind to mast cells, basophils, and blood platelets to trigger the release of mediators, e.g., histamine, which increases blood vessel permeability and smooth muscle contraction of the bronchioles leading of bronchospasm. C5a also functions as a powerful chemotactic factor that attracts phagocytes to the site of complement fixation [8].

**Cytokines** are soluble chemical messengers by which cells of the immune system communicate with each other. The most important cytokines are: ***interferon, interleukins (Il), cytotoxins (tumour necrosis factor).*** Cytokines which regulate communication between leukocytes and other cells named Interleukin - a name chosen to describe their function of communication between white cells.

Conformity of cytokine regulation:

- The same cytokine can be synthesized by different cells, the same cell can synthesize different cytokines
- The same cytokine can stimulate and suppress the target cell activity
- The simultaneous effect of several cytokines on the target cell can be both synergistic and antagonistic
- Cytokines can interact with the receptors released out of the cell, thus inhibiting the contact of cytokines with the target
- Cytokines act in low concentrations 0,001 microgram/milliliter
- For action of cytokines it is enough to be combined with 10 per cent of cell receptors.

**Table 5-1 Classification of cytokines**

| № | By the source: | By the participation in immune mechanisms: |
|---|---|---|
| 1 | **Lymphokines** *(Lymphocytes)* | **Promotion of inflammation** IL-1; IL-6; IL8; ά THF, Interferon-stimulation of innate non-specific defense, inflammation and development of specific immune reactions |
| 2 | **Monokines** *(Monocytes, Macrophages)* | **Anti-inflammatory** IL-4; IL-13: They inhibited non-specific and specific immune reactions |

**Interferons** are a class of similar antiviral proteins produced by certain animal cells after viral stimulation (or after exposure to other inducers). One of the principal functions of interferons is to interfere with viral multiplication. The next feature of interferons is that they are host-cell-specific but not virus specific. This means that interferon produced by human cells protects human cells, but not other cell. Other animals cannot be used as a source of interferons for human therapy. Rather, the genes for human interferons have been cloned and material for medical trials is now produced by genetic engineering techniques. However, the interferon of a species is active against a number of different viruses.

Human interferons are of **three types**:

- Alpha interferon **(α)**
- Beta interferon **(β)**
- Gamma interferon **(γ)**

**α interferon** is produced by leukocytes and possess mainly antiviral effect and after that anticancer (inhibit cancer cells), antiproliferative effect**.**

**β interferon,** produced by the fibroblasts in connective tissue, possess mainly anticancer effect and then antiviral effect.

**γ interferon** produced by lymphocytes and have high immunomodulate effect and faint (poor) antiviral effect.

All interferons are small proteins, with molecular weights between 15000 and 30000. They are quite stable at low pH and fairly resistant to heat.

Produced by virus-infected host cells only in very small quantities, interferon diffuses to uninfected neighbouring cells. It reacts with plasma or nuclear membrane receptors, inducing the uninfected cells to manufacture mRNA for the synthesis of antiviral proteins (AVPs). These proteins are enzymes that disrupt various stages of viral multiplication. For example, one AVP inhibits translation of viral mRNA by blocking initiation of protein synthesis. Another inhibits polypeptide elongation. Still another is involved in destroying viral mRNA before translation. The low concentrations at which interferon inhibit viral multiplication are nontoxic to uninfected cells. Interferon is effective for only short periods. It typically plays a major role in infections that are acute and short term, such as cold and influenza. Another problem is that it has no effect on viral multiplication in cells already infected. They inhibit the growth of viruses by blocking the translation of viral proteins. Because interferons are produced within a few hours of the initiation of viral replication, they may act in the early phase of viral diseases to limit the spread of virus [4, 5].

Interferon used to treat a variety of blood cancers, and solid tumours. For rising antiviral protection, we use interferences which raise the production of interferons.

**The main peculiarities of interferons are:**

- Universality- are not virus specific (**IFN** are active against all viruses)
- Expressed species activity
- Biological activity of **IFN** is depends on polypeptide component
- Species specificity is depends on saccharide portion
- Residual affection of **IFN** is detected after washing of preparations
- Absence of toxic action
- High effective action (minimal doses of **IFN** -s can leave antiviral action).

**Cytotoxins** - macrophages produce tumour necrosis factor **(TNF)** which is an important inflammatory mediator. Low concentrations of it is activates

neutrophils and increases their adhesion toendothelial cells. High concentration mediates septic shock, acts as cachectin, causes necrosis of tumours [5].

**4. Illustrative material:** tables, charts, posters, multimedia projector.

**5. Literature:**

1. Turvey S.E, Broide D.H. Innate immunity. J Allergy Clin Immunol. 2010;125(Suppl 2): S24–32.

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8. N. Parker, M. Schneegurt, Anh-Hue Thi Tu, B.M. Forster, P. Lister, - Microbiology, textbook, - Houston, Texas: 2017 Rice University, - 4.0 International License (CC BY 4.0).

**6. Checklist:**

1. Types of immunity.

2. Who was the first person studied the body's response to foreign substance?

2. The main stages of phagocytosis.

3. How many types have human interferons?

4. Functions of the complement system.

5. Cells of the immune system.

6. The main effects of complement.

7. How classified cytokines?

8. Central and peripheral lymphoid organs.

9. What is the immune system?

10. What is the natural killer cells?

## LECTURE № 6.

## 1. TOPIC: ANTIGENS. ANTIGEN-PRESENTING CELLS. ANTIBODIES. THE SYSTEM OF CELLULAR IMMUNITY

**2. Purpose:** To characterize antigens and antibodies, antigen-presenting cells. To acquaint students with their role in induction and regulation of immune response. Define the main functions of the T-system.

**3. Abstracts of the lecture**

Unlike nonspecific immunity, specific immunity is eventually formes only during the body's response to genetically foreign agents and is an evolutionarily later protective adaptation. The specific reactions of the organism are call immune reactions. All immune reactions are basing on mechanisms specific for specific antigens, which recognized by protein structures - antibodies complementary to antigens (i.e. spatially corresponding to them as an imprint of the original) [1].

The term "antigen" was form from the words "antibody" and "generator. Thus, originally, an antigen was consider a substance that causes the production of antibodies. However, with a deeper understanding of the mechanisms of the immune response, the following definition is appropriate for an antigen.

"The antigen, once in the host's body, causes the formation of specific antibodies and T-lymphocytes that react against the antigen."

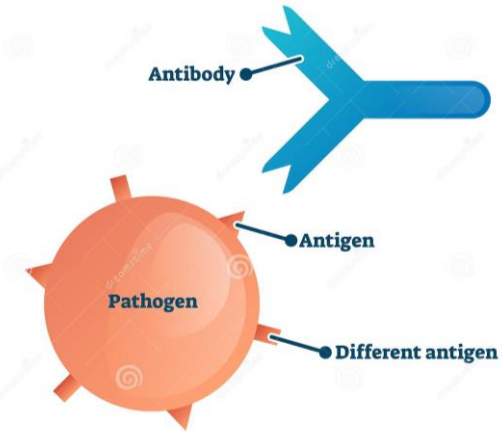



**Figure 6.1. Antigen**

## IMMUNOGENICITY AND ANTIGENICITY

Antigens have two very important characteristics: immunogenicity, or the capability to stimulate a specific immune response, and antigenicity, the capability to react specifically with antibodies. An antigen that possesses both of these characteristics called a **complete antigen**. An antigen that is reactive but not immunogenic named an **incomplete antigen or haptein** (*haptein*: to grasp). A hapten made into a complete antigen by connecting it with a larger carrier molecule, such as a protein [2].

## DETERMINANTS OF ANTIGENICITY

A number of factors have been identified that make a substance immunogenic. Some of the important determinants of antigenicity include:

1. Molecular size
2. Foreignness
3. Chemical-structural complexity
4. Stability
5. Other factors.

**Molecular Size**

In general, protein molecules with large molecular weight are highly antigenic. Substances with molecular weights of about 100,000 Da and more are highly immunogenic, while substances with molecular weights of less than 5000 Da are generally not immunogenic. This property has been exploited in experimental studies by using high molecular weight proteins like bovine gamma globulin (MW 150,000 Da) to induce an immune reaction. Substances with low molecular weight may be made antigenic by adsorbing these on carrier particles, such as bentonite, kaolin, and other inert particles.

**Foreignness**

To be immunogenic, a molecule must be recognized as nonself i.e., foreign. The molecule is considered self or nonself by the immune system depending on whether or not the molecule was exposed to the immune system during fetal development.

***Foreignness*** implies ability of the host to tolerate self-antigens. Tolerance to self-antigens develops by contact with them in the initial phases of the development of immune system, particularly during the development of lymphocytes.

In general, the more distantly related two species are, the greater the immunogenicity of a molecule from one species will be when exposed to the other. For example, the bovine serum albumin is more immunogenic in a chicken than in a goat. A graft from an unrelated human will be rejected within about 2 weeks unless immunosuppressive drugs are used, but a graft from a chimpanzee will be rejected within hours even if drugs are used. In contrast, a kidney graft from an identical twin will be accepted readily.

**Chemical-Structural Complexity**

Proteins are the most potent immunogens followed by polysaccharides. Nucleic acids and lipids are not efficient in eliciting a good immune reaction, although they may act as haptens. Structural complexity of a protein contributes to its immunogenicity. Chains of single amino acids or single sugars are poorly immunogenic, but if different amino acids or sugars are combined in the same molecule, the immunogenicity is greatly enhanced.

In cell-mediated immunity, the response of T cells to the peptide component of the proteins depends on how the peptide is recognized and presented by the MHC cells. Therefore, the structure of protein plays an important role in its immunogenicity, especially in inducing cellular immunity.

The lipid-specific antibodies are not easily produced; hence, they do not play a major role in immunity. However, these antibodies have a role in the

measurement of certain lipid-based molecules and drugs. These antibodies are produced first by treating lipids with haptens and then conjugating with suitable carrier molecules, such as the proteins (e.g., hemocyanin or bovine serum albumin).

**Stability**

Highly stable and nondegradable substances (e.g., some plastics, metals, or chains of D-amino acids) are not immunogenic. This is because internalization, processing, and presentation by antigen-presenting cells (APCs) are always essential to mount an immune response. Therefore, very stable substances (such as silicon) have been successful as nonimmunogenic materials for reconstructive surgeries, such as breast implants.

On the other hand, if a substance is very unstable, it may break up before an APC can be internalized, and hence become immunogenic. In addition, large, insoluble complexes are more immunogenic than smaller, soluble ones. This is because macrophages find it easier to phagocytose, degrade, and present the insoluble complexes.

**Other Factors**

**Biological system**

The biological system also plays an important role in determining the immunological efficacy of an antigen. Some substances are immunogenic for some people but not for others (i.e., responders and non-responders). It is happens because individuals may be missing or have altered genes encoding receptors for antigen on B cells and T cells, or they may be missing the corresponding genes required by APCs to present antigen to helper T cells (TH) [3].

**Dosage and route of the antigen**

The dose of antigen and the route by which it comes into contact with the immune system also influence immunogenicity of the antigen. Very low doses of antigen do not stimulate immune response, either because too few lymphocytes are contacted or because a nonresponsive state is elicited. Conversely, an extremely

high dose also fails to elicit tolerance. Repeated administration of antigens (booster doses) may be required to enhance immune response of the host to certain antigens. This is particularly important in case of vaccines where a prerequisite immune level needs to be attained. Hence the booster doses of vaccines, such as DPT (Diphtheria, Pertussis, Tetanus), DT (Diphtheria, Tetanus), etc., are given to ensure good protective levels of antibodies. Generally, antigens are administered by the parenteral route to produce good level of antibodies. The antigens can be given by (a) intravenous, (b) subcutaneous, (c) intradermal, (d) intramuscular, (e) intraperitoneal, and (f) mucosal routes. Usually, the subcutaneous route of administration proves to be better than intravenous routes at eliciting an immune response.

**Adjuvants**

Adjuvants are the substances that when mixed with an antigen and injected with it boost the immunogenicity of the antigen. Adjuvants increase both the strength and the duration of immune response. Adjuvants boost immunogenicity of antigens in several ways:

Adjuvants like aluminum potassium sulfate (alum) and Freund's water-in-oil adjuvant prolong the persistence of antigen by forming a depot at the injection site. Alum precipitates the antigen and releases it a little at a time. The water-in-oil emulsion forms small droplets with the antigen and also releases these slowly over time.

Freund's complete adjuvant contains, in addition to the emulsifying factors, heat-killed mycobacteria. The bacterial components activate macrophages and increase both the production of IL-1 and the level of B7 membrane molecules, which enhances the immune response. The increased expression of class II MHC increases the ability of APC to present antigen to TH cells. B7 molecules on the APC bind to CD28, a cell-surface protein on TH cells, triggering stimulation, an enhancement of the T-cell immune response.

Some adjuvants, like synthetic polyribonucleotide's and bacterial lipopolysaccharides, stimulate nonspecific lymphocyte proliferation and bring about their action [3].

**Antigen Nomenclature**

Antigens that require T-cell involvement for an immune response to occur named *T-cell-dependent (TD) antigens*. Antigens that stimulate B cells without the involvement of T cells named *T-cell-independent (TI) antigens.* The booster or memory response mediated through T cells and because can only initiated by TD antigens.

An autologous antigen is an internal antigen that produces autoantibodies under appropriate circumstances. Thus, autologous antigen is synonymous with auto- or self-antigen.

***Autologous Antigen***

An autologous antigen is an internal antigen that produces autoantibodies under appropriate circumstances. Thus, autologous antigen is synonymous with auto- or *self-antigen*.

***Heterologous Antigen***

A heterologous antigen is simply an antigen other than the one used for immunization; it may or may not react with the antiserum depending on its chemical similarity to the homologous antigen.

***Homologous Antigen***

The homologous antigen is the antigen applied in the production of the antiserum.

***Isophile Antigen***

The isophile antigens or *isoantigens* are molecules from one individual of one species that are antigenic to another member of the same species. The best example of isoantigens is the blood group system.

**Cross Reactivity of Antigens**

An antigen can be a complex mixture of many antigenic molecules, such as a microbe. Cross-reactivity can occur if different complex antigens have similar antigenic molecules.

Cross-reactivity can also result from the presence of different molecules in the preparation, some of which are share. Because immunological reactivity is direct against small parts of molecules (epitopes) rather than the entire molecule, cross-reactivity can occur because some epitopes on a given molecule may be similar and others may be different. Consequently, immune sera raised against a drug containing only one type of molecule can react with preparations of different molecules in the presence of common epitopes.

**Antigenic Determinant Sites (Epitopes)**

The entire antigen does not induce an immune response. Only a limited portion of the antigen molecule is an inducer of B- and T-cell response. This is the part of the antigen to which, the antibody or T-cell responds. It is call the antigenic determinant region or *epitope* (Figure 6.2).

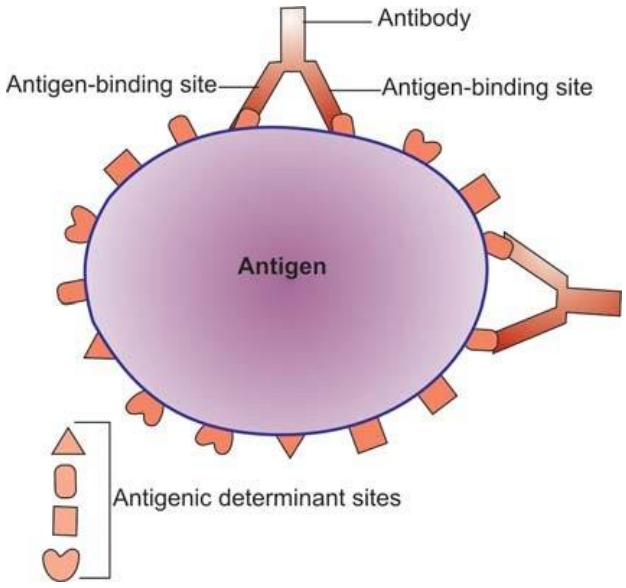



**Figure 6.2. Antigenic determinant sites**

**Haptens**

Haptens are too small molecules to be antigens themselves. Injection of a hapten into an animal usually does not induce an immune response. At the same time, a hapten is capable of reacting with an antibody induced by the introduction of a hapten-carrier complex. Thus, a hapten is define as a molecule that is not immunogenic on its own, but can react with a pre-formed antibody of the desired specificity.

Haptens can covalently combined with existing established antigens (carriers) to create new antigenic determinants. These hapten-antigen (carrier) complexes, or conjugated or neoantigens, generate antibodies with specificity to groups of haptens. Haptens come in two types:

a. *Simple haptens* combine with specific antibody but do not produce any antigen-antibody product viz precipitation.

b. *Complex haptens* do combine with specific antibody to produce precipitates because of presence of multiple antibody combining sites on its surface [3, 4].

**Superantigens (SAgs)** are a class of antigens that cause excessive activation of the immune system. SAgs produced by certain pathogenic viruses and bacteria, most likely as a defense mechanism against the immune system. Unlike a normal antigen that activates one or more helper T cells (0.00010.001% of the body's T cells are activated), these SAgs are capable of activating up to 20% of the body's T cells. A large number of activated T cells secrete a large number of cytokines, the most important of which is interferon gamma. This excessive amount of IFN-gamma, in turn, activates macrophages. Activated macrophages, in turn, overproduce pro-inflammatory cytokines such as IL-1, IL-6 and TNF-alpha. TNF-alpha is particularly important as part of the body's inflammatory response. Under normal conditions, it is release locally at low levels and helps the immune system defeat pathogens. However, when it is systemically released into the blood in high concentrations (due to mass

activation of T cells as a result of SAg binding), it can induce severe and life-threatening symptoms, including shock and multi-organ failure [4].

## ANTIGENS OF HUMAN ORGANISM

**Isoantigens:** are those substances which have antigenic properties and are contained in some individuals of a given species. They have been found in the erythrocytes of animals and man. And on the basis of antigenic structure the erythrocytes of all people can be subdivided into four groups (O; A; B; AB). These data are taken into account during blood transfusion.

**Autoantigens:** are substances capable of immunizing the body from which they are obtained. Thus, they become modified and are capable of bearing an antigenic function. These substances include the eye lens, spermatozoids, liver, lungs and other tissues. Under ordinary conditions they do not come in contact with the immunizing system of the body, therefore antibodies are not produced against such cells and tissues. However, if these tissues are injured, then autoantigens may be absorbed, and may cause the production of antibodies which have a toxic effect on the corresponding cells. The origination of autoantigens is possible under the influence of cooling, radiation, drugs, virus infections, bacterial proteins and toxins of streptococci, staphylococci, tubercule bacilli and other factors. The production of autoantigens is the result of the disturbance of species specificity which provides for the antigenicity of a number of substances found in the given body.

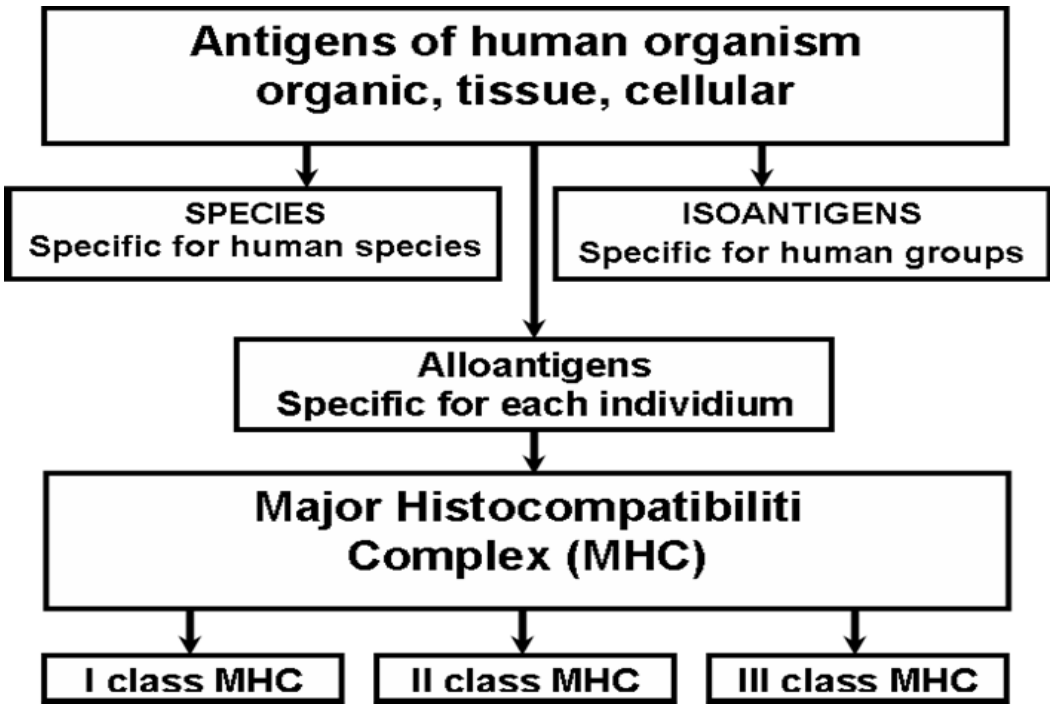



**Figure 6.3. Antigens of human organism**

**Alloantigens: Histocompatibility antigens** which subdivided into three groups:

**Class I MHC (Major Histocompatibility Complex) proteins –** these are glycoproteins found on the surface of virtually all nucleated cells. They play role of transplant antigen.

The main biological role of MHC I is recognition of self and non-self. Cytotoxic T cells respond to antigen in association with class I MHC proteins.

**Class II MHC proteins –** These are glycoproteins found on the surface of certain cells (professional APC), including macrophages, B cells, dendritic cells of the spleen, and Langerhans cells of the skin. Helper T-cells recognize class II proteins.

**Class III MHC proteins -** include some components of the complement system and a few others cytokines.

MHC genes and proteins are also important because many autoimmune diseases occur in people who are carriers of certain MHC genes, and that the success of organ transplants is largely determined by the compatibility of the

donor and recipient MHC genes.

**Tumour antigens –** are result of malignant transformation of cells.

**Singen antigens** –antigens of different individuals, which are not differ [2, 4].

## ANTIGENIC STRUCTURE OF THE MICROBIAL CELL

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

Bacterial antigens are:

- **H-antigen** (flagellar) which thermo labile and are destroyed at a temperature of 56-80$^0$C. Only flagellated organisms have H antigen (E. coli, S. typhi).
- **O-antigens** (somatic) the cell wall antigen. It is the outer polysaccharide portion of the lipopolysaccharide. O antigen is the basis for the serologic typing of many enteric rods. Somatic antigens are thermo resistant and withstand heating to 80-100$^0$C.
- **Vi-antigens-** a relatively thermolabile antigen was isolated from virulent strains of the typhoid bacillus. Possess high virulence property and named virulence antigen.
- **K-antigen:** capsular or polysaccharide antigen. They are formed at the expense of the O-antigens and are located on the surface of the cells. The K antigen contain thermo-labile L-and B-antigens and thermo-resistant A antigen. K antigen located superficial to O antigen, masking O antigen. K antigen contains acidic polysaccharides such as glucoronic acid, galacturonic acid.
- **Protective** antigens have been found in exudates of animals suffering from anthrax.

Microbial **toxins** also have antigenic properties. Rendered harmless by formalin and heat treatment, exotoxins lose their toxic properties and almost

completely retain their antigenic functions. They are known as anatoxins, and are widely used in immunizing people against diphtheria and tetanus. Bacterial **enzymes** are complete antigens too.

**Heterogenic antigen** (Forssman antigen): There are antigens (haptens) found in different species of animals (e.g., heterogenic antigens are contained in the protein structure of organs of guinea pigs, in the erythrocytes of sheep and in salmonellas etc.).

When antigenic structures of the host (human organism) are similar to those of the causative agent, the micro-organism is incapable of producing immunity, as the result of which the disease follows a graver course. Such a condition is called **antigenic mimicry** (e.g., human erythrocytes have antigens common with staphylococci, streptococci and other causative agents of infectious disease).

**Cross reacting antigen (CRA) –** discovers in microorganisms and in human tissues (hemolytic streptococci of A group contains CRA which is common with autoantigens of myocardium and kidney glomeruli and they can cause myocarditis and glomerulonephritis).

**Antigenic structure of viruses:** For simple, viruses antigenic structure connected with nucleocapsid. By chemical structure they are ribonucleoprotein either deoxyribonucleoprotein which are soluble and signified **S-antigen** (solution). At complex (enveloped) viruses antigenic structure connected with nucleocapsid and glycoproteins of external membrane. Many viruses contain peculiar (specific) superficial (surface**) V (viral)-antigens** which are hemagglutinin and neuraminidase [4, 5].

## ANTIGEN PRESENTING CELLS

**Macrophages -** in contrast to T cells, B cells, and NK cells, which differentiated from lymphoid stem cells, macrophages arises from myeloid

precursors. Macrophages have three main functions: **a) phagocytosis, b) antigen presentation, c) cytokine production**. They take part in immune response, due to macrophages foreign material is ingested and degraded, and fragments of antigen are presented on the macrophage cell surface (in conjunction with class II MHC molecules) for interaction with the TCR (T cell receptor).

**Dendritic cells** they are close to macrophages, but they haven't phagocytic property. They possess II MHC molecules and they have antigen fixation property. They formed antigen-MHC complex and present it to T lymphocytes. They have I MHC molecules too and can present antigen to the CD8 cells and initiate cytotoxic reaction.

**B lymphocytes** are antigen presenting cells, they interact with antigen over the specific receptors, after antigen undergo an endocytosis. After few hours antigen expressed on the surface of B cell in complex with II MCH cells further B- lymphocytes enter into contact with T cells and activate them. APCs are endothelial cells, fibroblasts, Langerhans cells, etc. [3, 5].

## ANTIBODIES-IMMUNOGLOBULINS

The production of antibodies is part of the immune system's response to antigenic stimulation. Antibodies are blood proteins, all of which are globulins (hence synonym: immunoglobulins) that are part of the serum gamma fraction. In addition to those found in the blood (humoral antibodies), some types of antibodies are fixed on cells or tissues of the body or exist in body secretions (cell-associated antibodies).

### Structure

Immunoglobulin (Ig) molecules are symmetrical structures. In solution, they are Y-shaped after binding to the antigen. Each molecule consists of four polypeptide chains: two equal heavy chains (H) and two equal light chains (L). They are

designated as light or heavy depending on their molecular weight, which is 50,000 to 70,000 daltons for the H chains and 20,000 to 25,000 daltons for the light chains (Figure 6.4). The L-chain is attached to the H-chain by a disulfide bond.

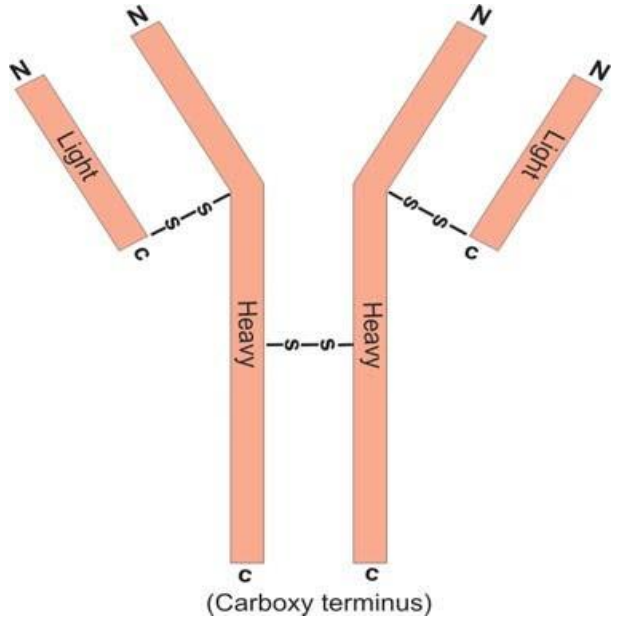



**Figure 6.4.** Antibody molecule

The two H-chains linked by 1 to 5 S-S-links, depending on the immunoglobulin class. The H-chains are structurally and antigenically different for each immunoglobulin class. The L-chains are similar in all classes of Ig. There are two types: kappa (κ) and lambda (λ). An immunoglobulin molecule can contain either a kappa or a lambda, but never both. The L- and H-chains divided into variable and constant regions. The L-chain consists of one variable domain (VL) and one constant domain (CL). Most H-chains consist of one variable domain (VH) and 3 or more constant domains (CH). Each domain is approximately 110 amino acids long. The variable domains are responsible for antigen binding, while the constant domains are responsible for biological functions. There are three extremely variable (hypervariable) amino acid sequences in the variable regions of both the L- and H-chains, which form the antigen-binding site [2, 4].

An antibody molecule can cleaved by papain to form two identical fragments, each with a single antigen-binding site. This fragment named the Fab fragment,

which binds to the antigen. The third fragment, which does not have the ability to bind to the antigen, named the crystallizable Fc-fragment (Figure 6.5.). Under experimental conditions, enzymes can used to separate the antibody into Fc- and Fab-fragments, which has several applications:

| ***Enzyme*** | ***Location of cleavage*** | ***First fragment*** | ***Second fragment*** |
|---|---|---|---|
| Papain | At hinge region | Two Fab fragments | Fc fragment |
| Pepsin | Below hinge region | One F(ab')2 fragment | Fc fragment |

. ***Fc-region***: The Fc-region (fragment, crystallized) originates from the "Y" stem and consists of two heavy chains that contribute two or three constant domains depending on the antibody class. The Fc domain binds to various cellular receptors and complement proteins. Thus, it mediates various physiological effects of antibodies, such as opsonization, cell lysis, degranulation of mast cells, basophils and eosinophils, and other processes.

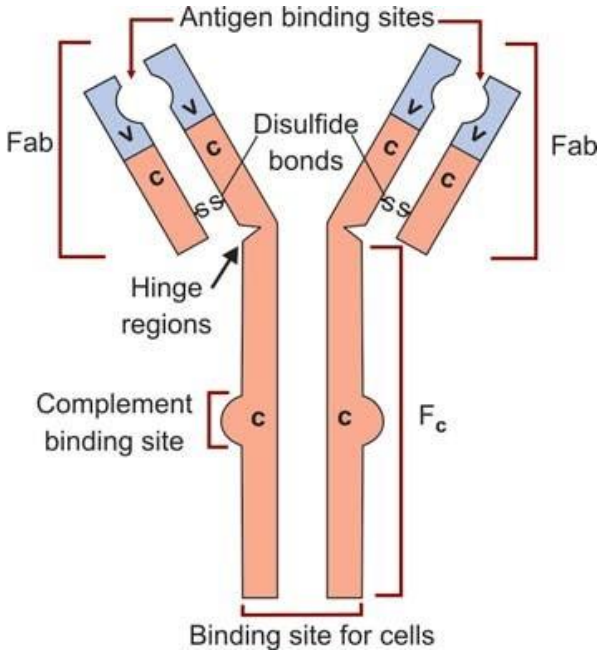



**Figure 6.5. Structure of Ab molecule**

- ***Fab region:*** Each end of the forked "Y" on the antibody called the Fab

region (*antigen-binding fragment*). It consists of one constant and one variable domain of each of the heavy and light chains. These domains form a paratope – an antigen binding site – at the amino-terminal end of the monomer. The two variable domains bind the epitope on their specific antigens.

***Functions of Fab and Fc***

The Fab- and Fc-fragments of the immunoglobulin molecule have various functions. The Fab-fragment binds to the antigen because it contains an antibody binding site. The Fab fragment binds to the antigen to form soluble complexes that do not precipitate. The Fc-fragment does not bind to antigen, but has sites that determine the effector functions of antibody molecules, such as complement fixation, binding to phagocytes, transplacental transmission, binding to mast cells, and secretion into body fluids.

## CLASSES OF IMMUNOGLOBULINS
## (Isotype of Ig)

Based on the structure of the constant heavy chain region immunoglobulins divided into basic groups called classes as well as isotypes. In humans there are five classes: immunoglobulin G, IgA, IgM, IgD and IgE. The heavy chains of these immunoglobulins designated by Greek letter as gamma (γ), alpha (α), mu (μ), delta (δ) and epsilon (ε) respectively. Within class IgG, based upon different distinctive heavy chains and differing functional properties, there are four subclasses: IgG1, IgG2, IgG3 and IgG4. Similarly, there are two subclasses each of IgA and IgM.

The basis of the structure of all classes of antibodies is a monomer composed of two heavy and two light chains, which form di- and polymers. The classes of immunoglobulins differ in the number of monomers, valency (the number of active antigen binding centers-monovalent bivalent, polyvalent), avidity, ability to pass through a placental barrier. Monovalent antibodies are considered **incomplete.**

**IgM-antibodies** are the first antibodies to appear in response to initial

exposure to an antigen. The main immunoglobulin produced early in the primary response. IgM has a pentamer structure consisting of five monomers. Because the pentamer has 10 antigen-binding sites (this is observed only with small haptens. With larger antigens, the effective valency falls to five); it is the most efficient immunoglobulinin agglutination, complement fixation, and other antibody reactions and is important in defence against bacteria and viruses. IgM antibodies are relatively short lived, disappearing earlier than IgG; their demonstration in serum indicates recent infection. It has the highest avidity of immunoglobulins. It is the earliest immunoglobulin to be synthesized by the fetus, beginning by about 20 weeks of age. It is not transported across the placenta. They make up 5-13 % of the antibodies in serum. IgM can activate complement. Monomeric IgM is the major antibody receptor on the surface of B lymphocytes for antigenrecognition [4, 5].

**IgG -** It is divalent because it has two identical antigen-binding sites (two L chains, two H chains). IgG antibodies account for about 80% of all antibodies. IgG is the predominant antibody in the secondary response and constitutes an important defence against bacteria and viruses, neutralize bacterial toxins. IgG is the only antibody to cross the placenta and confer passive immunity to a fetus; only its Fc portionbinds to receptors on the surface of placental cells. It is therefore the most abundant immunoglobulin in newborns. IgG can activate complement, and when bound to antigens, enhance the effectiveness of phagocytic cells. IgG subdivided into four classes IgG1-IgG4, based on antigenic differences in the H chains and on the number and location of disulfide bonds. IgG1 makes up most (65%) of the total IgG. IgG2 antibody is directed against polysaccharide antigens and is an important host defence against encapsulated bacteria.

**IgA** - accounts about 10-15% of the antibodies in serum. IgA is the main immunoglobulin in secretions such as colostrum (its presence in colostrum probably helps to protect infants from gastrointestinal infections especially),

saliva tears, and respiratory, intestinal and genital tract secretions. It consists of two parts: Serum and secretory. The main function of secretory IgA is to prevent attachment of micro-organisms eg, bacteria and viruses, to mucous membranes. It ensured local immunity. SIgA can activate complement by alternative pathway, which stimulated local phagocytic response. Serum IgA circulates in the serum mostly as a monomer [1, 3].

**IgD – is monomer,** makes up only about 0.2 % of the total serum antibodies. This immunoglobulin has no known antibody function but may function as an antigen receptor; it is present (with IgM) on the surface of many B lymphocytes and serve as recognition receptors for antigens.

**IgE –** they constitute 0.002 % of the total serum antibodies IgE mediates immediate (anaphylactic) hypersensitivity; it participates in host defences against certain parasites (Helminths). The Fc-region of IgE interact to the surface of mast cells and basophils. Bound IgE serves as a receptor for an antigen (allergen), and this antigen-antibody complex causes an immediate (anaphylactic) allergic reaction through the release of mediators. In people with allergic reactivity, the amount of IgE is significantly elevated, and it can appear in external secretions. IgE does not fix complement and does not cross the placenta.

IgE is the main host defence against certain important Helminth infections, such as Trichinella, Ascaris, and the hookworms. The serum IgE level is usually increased in these infections. Because the worms are too large swallowed by phagocytes, they are killed by eosinophils, which release enzymes that destroy the worms. IgE, specific to worm proteins, binds to receptors on eosinophils, triggering an antibody-dependent cellular cytotoxicity response.

Because immunoglobulins are proteins, they are antigenic, and that property allows them to be subdivided into isotypes, allotypes and idiotypes.

**Isotypes** are defined by antigenic (amino-acid) differences in their

constant regions. For example, IgG and IgM are different isotypes; the constant region of their H chains is different antigenically (the five immunoglobulin classes – IgG, IgM, IgA, IgE, IgD- are different isotypes; their H chains are antigenically different.

**Idiotypes** are the antigenic determinants formed by the specific amino acids in the hypervariable region. Each idiotype is unique for the immunoglobulin produced by a specific clone of antibody-producing cells. Anti-idiotype antibody reacts only with the hypervariable region of the specific immunoglobulin molecule that induced it [1, 3, 4].

Although antibody formation usually involves helper T cells, certain antigens, eg, bacterial polysaccharides can activate B cells directly, without the help of T cells, and are called T cell-independent antigens.

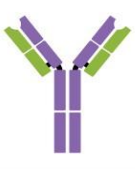
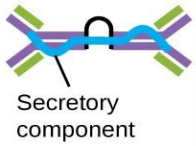
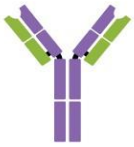
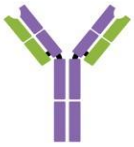
| The Five Immunoglobulin (Ig) Classes | | | | | |
|---|---|---|---|---|---|
| **Properties** | **IgG monomer** | **IgM pentamer** | **Secretory IgA dimer** | **IgD monomer** | **IgE monomer** |
| Structure |  |  |   |  |  |
| Heavy chains | γ | μ | α | δ | ε |
| Number of antigen-binding sites | 2 | 10 | 4 | 2 | 2 |
| Molecular weight (Daltons) | 150,000 | 900,000 | 385,000 | 180,000 | 200,000 |
| Percentage of total antibody in serum | 80% | 6% | 13% (monomer) | <1% | <1% |
| Crosses placenta | yes | no | no | no | no |
| Fixes complement | yes | yes | no | no | no |
| Fc binds to | phagocytes | | | | mast cells and basophils |
| Function | Neutralization, agglutination, complement activation, opsonization, and antibody-dependent cell-mediated cytotoxicity. | Neutralization, agglutination, and complement activation. The monomer form serves as the B-cell receptor. | Neutralization and trapping of pathogens in mucus. | B-cell receptor. | Activation of basophils and mast cells against parasites and allergens. |

**Figure 6.5. Types of Immunoglobulin**

**Dynamics of antibody formation.** When an individual first encounters an antigen, antibodies to this antigen are detected in the serum for several days or weeks, daccording to the nature and dose of the antigen and the method of administration (for example, orally, parenterally). The synthesis of antibodies is divided into ***primary*** and ***secondary***. The primary response occurs at the first encounter with the antigen. Secondary response – when there is a repeated encounter with the same antigen or a closely related (or cross-reacting) antigen months or years after the primary response (the secondary response is also called a memory reaction).

The kinetics of antibody synthesis in the primary and secondary response has gone through several stages: latent (lag), logarithmic (log), plateau and decline.

**A lag phase -** during which no antibody is detected. During this phase takes place microbial (antigen) distraction and its presentation to immune competent cells.

**A log phase** when the antibody titer increases logarithmically. Antibody is detected in lymph andblood.

**Plateau phase** during which the antibody titer stabilizes. The antibody level during this phase isarriving at maximum.

**A decline phase,** during which the antibody is cleared or catabolized.

An examination of the responses following primary and secondary antigenic challenge shows thatthe responses differ in four major respects:

**Time course**- Secondary response has a shorter lag phase (hours or 1-2 days) and an extend plateau and decline. In a primary response lag period is longer 3-5 days.

**Antibody titer** – The plateau levels of antibody are much greater in the secondary response, typically 10-fold or more than plateau levels in the primary response.

**Antibody class** – IgM antibodies form a major proportion of the primary

response, whereas the secondary response consists almost entirely of IgG, with very little IgM.

**Antibody affinity**- The affinity of antibodies in the secondary response is usually much higher.

This is referred to as 'affinity maturation'.

Memory cells (T and B), as the name implies, endow our host defences with the ability to respond rapidly and vigorously for many years after initial exposure to a microbe or other foreign material. This memory response to a specific antigen is due to several features:

**1.** many memory cells are produced, so that the secondary response is greater than the primary response, in which very few cells respond;

**2.** memory cells live for many years or have the capacity to produce themselves;

**3.** memory cells are activated by a smaller amounts of antigen and require less co-stimulation than do naive, inactivated T cells;

**4.** activated memory cells produce greater amounts of interleukins than do naive T cells when they are first activated [1, 3, 5].

**Immunology** was originally considered a protective process, helping the body to overcome infectious agents and their toxins. Immune response sometimes can give rise to an excessive or inappropriate reaction. This is referred to as immunodeficiency, autoimmune diseases, hypersensitivity.

**Autoimmunity** is a condition in which structural or functional damage results from the action of immunologically competent cells or antibodies against normal body components. The adult host usually exhibits tolerance to tissue antigens present during fetal life that are recognized as "self". However, in certain circumstances tolerance may be lost and immune reactions to host antigens may develop, resulting in autoimmune diseases. The most important step in the production of autoimmune disease is the activation of self-reactive helper (CD4) T cells. These self-reactive Th-1 or Th-2 cells can induce either

cell-mediated or antibody-mediated autoimmune reactions, respectively [5].

The following three main mechanisms for autoimmunity have been proposed.

1. **Molecular mimicry:** Various bacteria and viruses are implicated as the source of cross-reacting antigens that trigger the activation of autoreactive T cells or B cells (e.g. relationship between the M protein of S. pyogenes and myosin of cardiac muscle and antibodies against certain M proteins cross- react with cardiac myosin leading the rheumatic fever) [1].

2. **Alteration of normal proteins**: Drugs can bind to normal proteins and make them immunogenic.

3. **Release of sequestered antigens:** Certain tissues, e.g., sperm, central nervous system, and the lens are sequestered so that their antigens are not exposed to the immune system. When such antigens enter the circulation accidentally, e.g., after damage, they elicit both humoral and cellular responses, producing a spermatogenesis, encephalitis and endophthalmitis, respectively.

**4. Illustrative material**: tables, charts, posters, multimedia projector.

**5. Literature:**

1. Atazhakhova M.G., IMMUNOLOGY: Training manual - Maykop: Publisher IB Kucherenko V.O. 2020. – 56 p

2. Rajesh Bhatia, Rattan Lal Ichhpujani - Essentials of Medical Microbiology: textbook for universities. – 4th edition, Jaypee Brothers Medical Publishers *Editorial Consultant:* Ms Peromila MA (English), 2008

3. Subhash Chandra Parija - Textbook of Microbiology and Immunology - 2nd Edition, Published by Elsevier, a division of Reed Elsevier India Private Limited: Elsevier, 2012.

4. Murphy K.M, Travers P, Walport M. Janeway’s immunobiology. 7th ed. New York: Garland Science; 2007.

5. N. Parker, M. Schneegurt, Anh-Hue Thi Tu, B.M. Forster, P. Lister, - Microbiology, textbook, - Houston, Texas: 2017 Rice University, - 4.0 International License (CC BY 4.0).

**6. Checklist:**

1. Classification of antigens.
2. Antibodies.
3. The classes of immunoglobulins, their main characteristics, differences and peculiarities.
4. Antigens of human organism.
5. Antigenic structure of the microbial cell
6. Antigen presenting cells
7. Immunogenicity and Antigenicity

# LECTURE № 7.

## 1. TOPIC: SEROLOGICAL REACTIONS

**2. Purpose:** Master the methods of serological diagnosis of infectious diseases.

**3. Abstracts of the lecture**

The reactions of antigens with antibodies are called serological or humoral, since the specific antibodies involved are always in the blood serum. The reaction between antibodies and antigens occurring in vivo can be reproduced in the laboratory for diagnostic purposes. Serological reactions of the immune system entered the practice of diagnosing infectious diseases in the late XIX – early XX century.

The use of immune system reactions for diagnostic purposes is based on the specificity of the interaction of the antigen with the antibody. The determination of the antigenic structure of microbes and their toxins made it possible to develop not only antigens and serums, but also diagnostic serum. Diagnostic immune serum obtained by immunizing animals (for example, rabbits).

These serums are used to identify the antigenic structure of microbes or exotoxins by obtaining serological reactions (agglutination, precipitation, complement binding, hemagglutination, passive, etc.). Diagnostic immune serum treated with fluorochrome is used for rapid diagnosis of infectious diseases using immunofluorescence [1, 2, 6].

## ANTIGENS - ANTIBODIES REACTIONS

The main types of antigen-antibody reactions and their various modifications are shown in Table 7-1. Characteristics that are common to the various antigen-antibody reactions are shown in Table 7-2.

Here are some important definitions to help better understand antigen-antibody reactions:

**Affinity.** Affinity of an antigen-antibody reaction refers to the intensity of attraction between antigen and antibody molecules.

**Avidity.** Avidity is the strength of the bond after formation of the antigen-antibody complex.

**Table 7–1. Antigen-antibody reactions**

| *Reaction/test* | *Modified test* |
|---|---|
| **Precipitation** | Immunoelectrophoresis<br>Immunoprecipitation |
| **Agglutination** | Latex agglutination<br>Indirect hemagglutination<br>Coaglutination, Coombs test |
| **Complement fixation**<br>**Neutralization** | Conglutination<br>Plaque assays<br>Measurement of LD |
| **Immunofluorescence** | Indirect immunofluorescence<br>Immunofluorimetric assay |
| **Enzyme immunoassay** | Enzyme linked immunosorbent assay |
| **Radioimmunoassay** | Immunoradiometric assay |

**Sensitivity.** Sensitivity refers to the ability of the test to detect even very small amounts of an antigen or antibody. A test can be called highly sensitive if it has no or minimal false negatives.

**Table 7–2. Specifications of antigen-antibody reactions**

| № | Specifications |
|---|---|
| 1 | A specific cross-reaction may also occur |
| 2 | The antigen-antibody combination is stable, but reversible |
| 3 | No denaturation of the antigen or antibody during the reaction |
| 4 | Binding occurs on the surface |
| 5 | Whole molecules react. |

**Specificity.** Specificity means the ability of the test to detect a reaction only between homologous antigen and antibodies and with no others. In a highly specific test, false positives are minimal or absent.

### Measurement of Antigen and Antibody

The various methods currently available for detecting and measuring antigen and antibody are presented in Table 7-1. The measurement is usually made in *units* or *titer* of measurement. The titer of serum antibodies is the largest dilution of serum, which gives the observed reaction with the antigen in a particular test. Similarly, the value of an antigen in terms of its titer can be determined in relation to a known serum [2, 3, 7].

## PRECIPITATION

This reaction occurs only when the *antigen is in a soluble form.* Such an antigen in contact with a specific antibody in a suitable medium leads to the formation of an insoluble complex that precipitates. This precipitate usually settles to the bottom of the container. If it does not settle and remains suspended in the form of floccules, this reaction is called flocculation. Flocculation can occur not only in liquid media, but also in a gel system. By electrically moving the antigen and antibody, precipitation can be accelerated. Precipitation also depends on the optimal NaCl concentration, a suitable temperature and an appropriate pH.

### Zone Phenomenon

Deposition occurs most rapidly and abundantly when the antigen and antibodies are in optimal proportions or an equivalent ratio. These proportions are constant for all dilutions of the same reagent. The above principle can be illustrated by the following experiment:

Install a series of test tubes, each containing a constant amount of antibodies. Add a decreasing amount of antigen to the test tubes in a row. Watch out for precipitation, which will begin in the form of a nebula (Figure 7.1.).

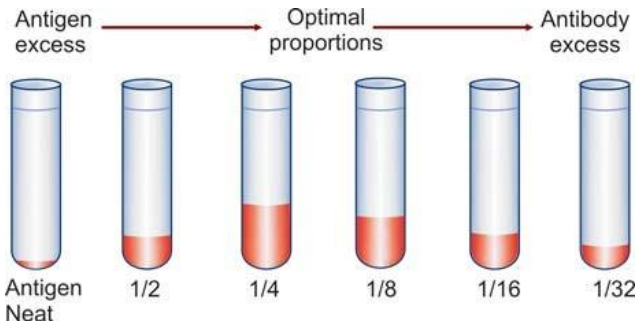



**Figure 7.1. Precipitation reaction**

Turbidity begins to appear in test tubes, gradually changing into visible aggregates or precipitates. It is observed that the amount of precipitate increases along the row, reaches a maximum when the antigen and antibody meet in optimal proportion, and then decreases as the concentration of antigen decreases.

If the number of precipitates in different tubes is plotted, the resulting curve will show three phases (Figure 7.2.).

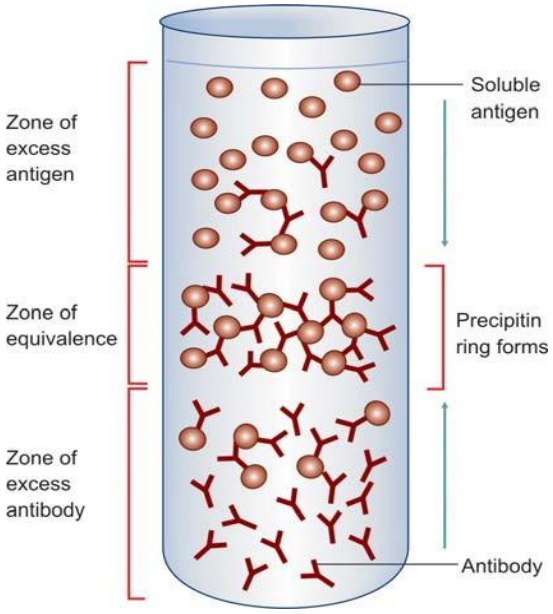



**Figure 7.2. Zone phenomenon**

**Zone of antibody excess.** Unbound antibodies should be present in this zone. This is called the antibody excess zone or prozone. Prozone is important in clinical serology because a serum sample containing large amounts of antibodies may give a false negative precipitation result until several dilutions have been tested.

**Zone of equivalence.** In this zone, both the antigen and the antibody are

completely deposited, and there is no unbound antigen or antibodies. The maximum fixation of complement also occurs in this zone.

**Zone of antigen excess.** In this zone, all antibodies have consolidated the antigen, and an additional non-combined antigen is present. In this zone, precipitation is partially or completely inhibited, since in the presence of an excess of antigen, soluble antigen-antibody complexes are formed [3, 4].

**Mechanism of Precipitation**

The lattice hypothesis in support of the deposition mechanism was put forward by Marrack in 1934. Large accumulations of antibodies and antigens are formed best when they are in optimal proportions. Under such conditions, after the initial unification of the molecules, free antigen binding sites and antigen-defining groups remain. They connect and help in the formation of lattices of antigen-antibody complexes (Figure 7.2. and 7.3.).

With an excess of the antibody, all the free determinants of the antigen molecule are soon absorbed by the antibody, and, consequently, the binding between the complexes is insignificant (Figure 7.3.) Similarly, in situations where the antigen is in excess, less precipitation occurs due to the inability of the antigen-antibody complexes to bind to other complexes and obtain a large filler or mesh [4, 5, 7].

The phenomenon of lattice formation is also true for agglutination.

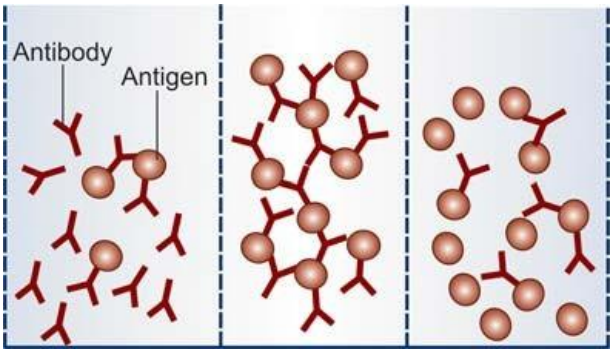



**Figure 7.3. Lattice formation**

**Applications of Precipitation Reactions**

Both qualitative determination and quantitative evaluation of the antigen, as well as antibodies, can be performed using precipitation tests. Qualitative tests are much more widely used for the detection and identification of antigens. It has been found that antigen detection using this method is more sensitive than antibody evaluation. Deposition tests are able to detect only 1µg of antigenic protein.

Below are some of the types of deposition and flocculation tests that have found application in diagnostic bacteriology:

**Ring test.** This is done by applying body extract over antiserum in test tubes. After some time, a ring of precipitation forms at the interface (Figure 7.4.). Typing of streptococci and pneumococci, a test for C-reactive protein and the detection of food adulteration are some of the applications of the ring test. This test also finds application in forensic medicine, where it is used to detect blood and semen stains.

**Slide test.** This is an example of flocculation, which observed when a drop of antigen and antibodies are mixed and shaken for some time. The reaction manifests itself in the form of flakes. VDRL one of the widely used tests for the diagnosis of syphilis.

**Tube flocculation test.** A flocculation test can also be performed in test tubes. The Kahn syphilis test is an example of this method, which is also used to standardize toxins and antitoxins.

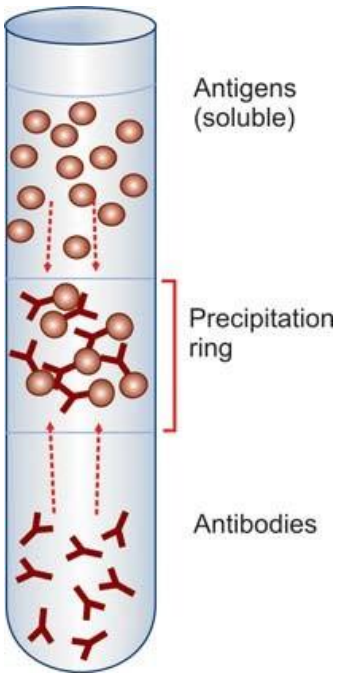



**Figure 7.4. Ring test**

**Immunodiffusion.** This term refers to the application of a gel that provides more sensitive and specific results. The reaction proceeds in the form of deposition bands and can be colored for a better view, as well as preservation. If a large number of antigens are present, each antigen-antibody reaction will result in a deposition line. This method also indicates identity, *cross-reaction*, and non-identity between different antigens. In practice, various types of immunodiffusion tests are used. They are based on the number of diffusions and the sizes in which they occur. The following four combinations can lead to:

***Single diffusion in one dimension.*** This is also known as the *Udine procedure*. An agar gel containing an antibody is placed in a test tube and a layer of antigen solution is applied to it. When the antigen migrates into the gel together with the antibody, the deposition line appears at the point where they meet in the optimal concentration. In the presence of various antigens, a large number of bands may appear (Figure 7.5.).

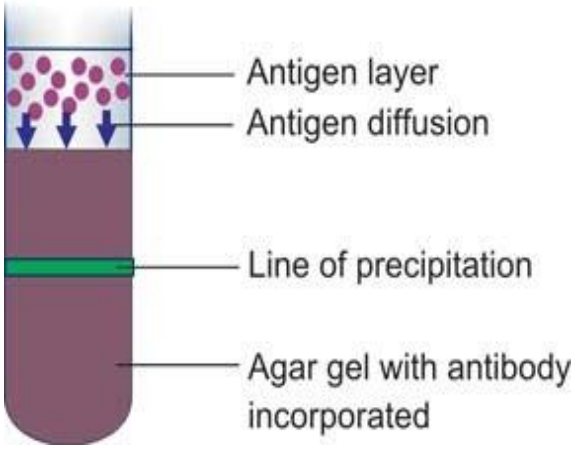



**Figure 7.5. Single diffusion in one dimension**

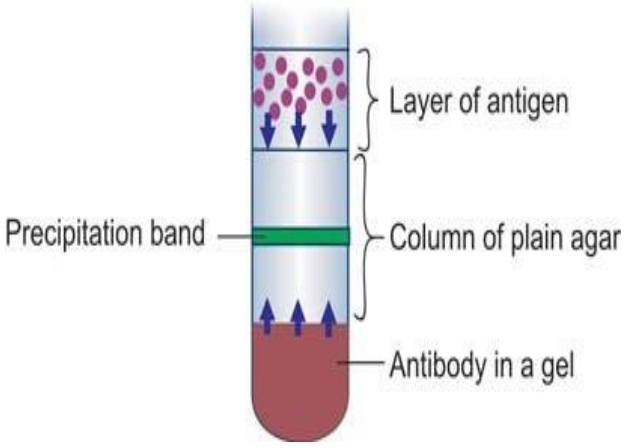



**Figure 7. 6. Double diffusion in one dimension**

***Double diffusion in one dimension.*** This is also named the *Oakley-Fulthorpe procedure*. After placing a certain amount of agar gel included with the antibody, a column of simple agar is added to the test tube. In addition, the antigen is applied in layers, as in the Udine procedure. The antigen moves down in the column of simple agar, and the antibody also migrates to the same column. A band of precipitation appears when they meet in an equivalent ratio (Figure 7.6).

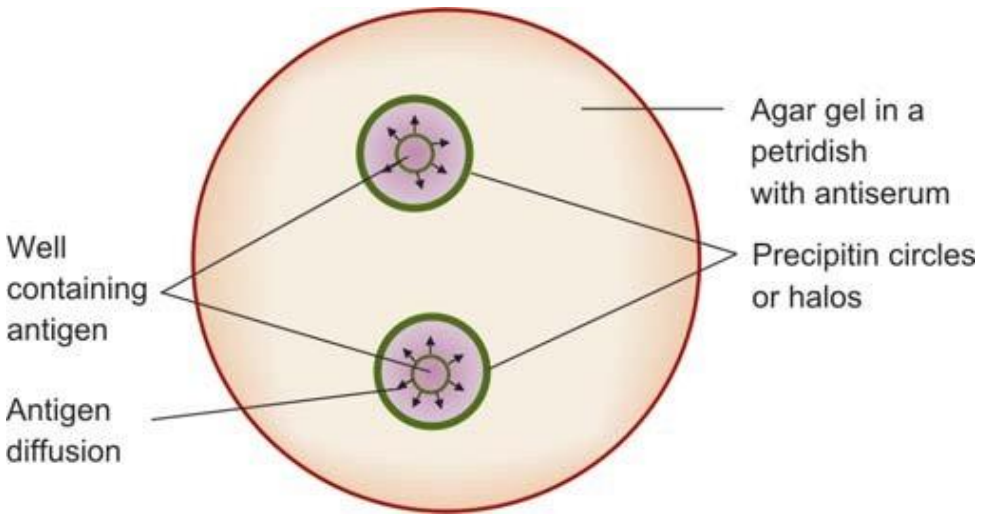



**Figure 7.7. Single diffusion in two dimensions**

***Single diffusion in two dimensions*** This procedure is also named *radiation immunodiffusion.* This is done in Petri dishes or slides having an agar layer in which the antibody is included. Wells are cut out in the agar layer, and antigen is added to these wells. The antigen diffuses, and concentric deposition bands can be seen around the well. The diameter of the deposition ring is used to estimate the antigen concentration (Figure 7.7.).

***Double diffusion in two dimensions.*** This method is better known as the *Ouchterlony procedure* and is the most widely used method that helps to directly compare different antigens and antiserums. The holes are cut into the agar layer in the Petri dish. Antiserum and antigen solutions are placed opposite each other in wells (Figure 7.8), and after several days of diffusion, deposition bands are formed where the antigen and antibody meet in a suitable proportion [3, 5, 6].

As shown in Figure 7.8. reactions with antigens C and D do not occur, since the antiserum in the central well contains antibodies only against antigens A and B. Identification lines formed between two wells A allow the method to be used to identify an unknown antigen (identification reaction). When neighboring antigens are not connected, the lines will intersect with each other (non-identity reaction). A cross-reaction or partial identity is indicated by the formation of a branch (partial identity reaction). A special type of this test is the Elek test for the toxigenicity of diphtheria bacilli.

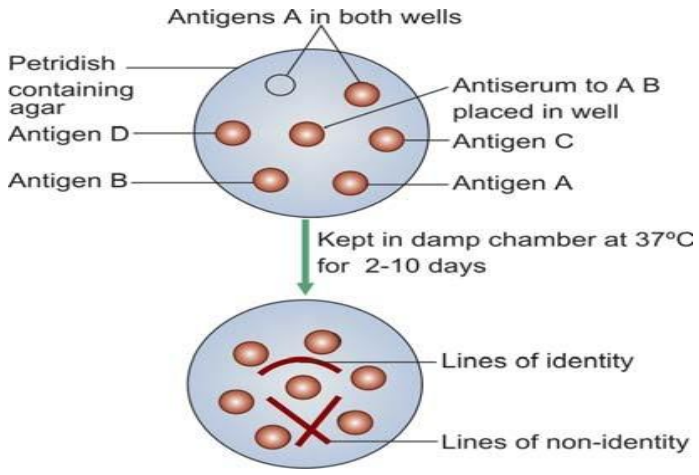



**Figure 7.8. Double diffusion in two dimensions**

***Immunoelectrophoresis.*** The use of electrophoresis in combination with immunodiffusion has significantly increased its usefulness. The antigen is first subjected to electrophoresis in agar. After electrophoresis, a trough is longitudinally cut out in the agar, and an antiserum against the electrophoresized antigen is placed in the trough. The two components diffuse towards each other, and precipitation bands form. For better visibility and safety, these stripes can be painted. The stages of immunoelectrophoresis are shown in Figure 7.9.

Electroimmunodiffusion is basically of two types:

***Cross immunoelectrophoresis.*** This is a one-dimensional single electroimmunodiffusion, which is also named Rocket electrophoresis because of the shape of the deposition bands. In this procedure, the proteins in the antigen are first separated by electrophoresis in an agarose gel, after which they are electrophoresized at right angles to the original direction into an antibody containing an agarose gel. The resulting sediment manifests itself in the form of sharp peaks. The peak height is determined by the concentration and mobility of the protein. This method also reveals the heterogeneity of antigens or the identity of different components by merging or overlapping patterns (Figure 7.10.).

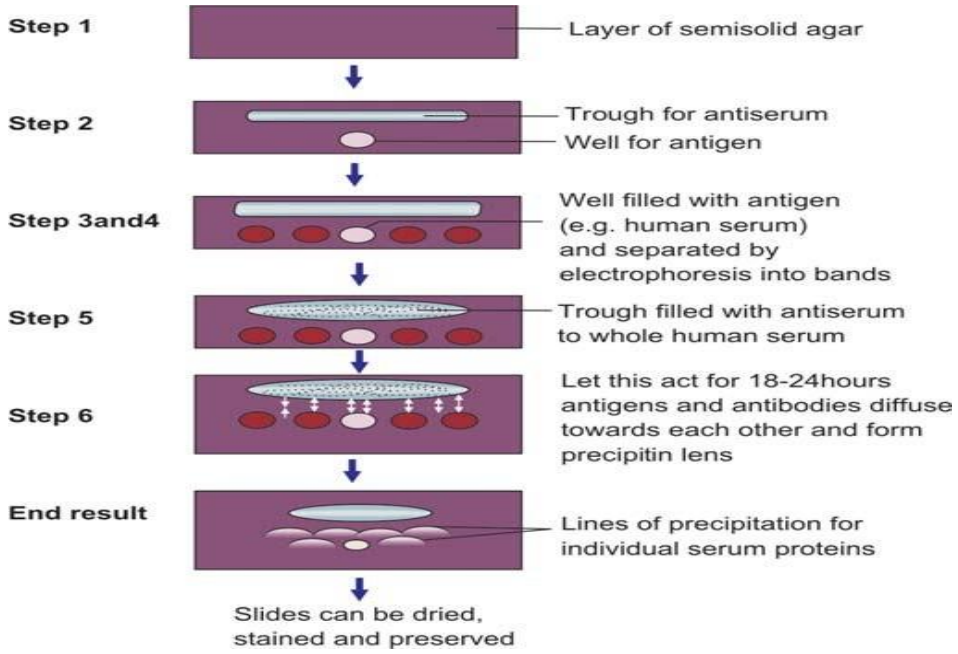



**Figure 7.9. Steps of immunoelectrophoresis**

***Counter current immunoelectrophoresis (CIE).*** In this procedure, the antiserum is located in one well, and the tested antigen is placed in another. At pH 8.2, the antibody moves to the cathode due to the electroosmotic flow, and if the well containing the antigen is located on the cathode side of the antibody well, the antigen, which usually moves to the anode, will meet the antibody somewhere between the two wells, and the deposition of the antigen-antibody complex will take place (Figure 7.11.).

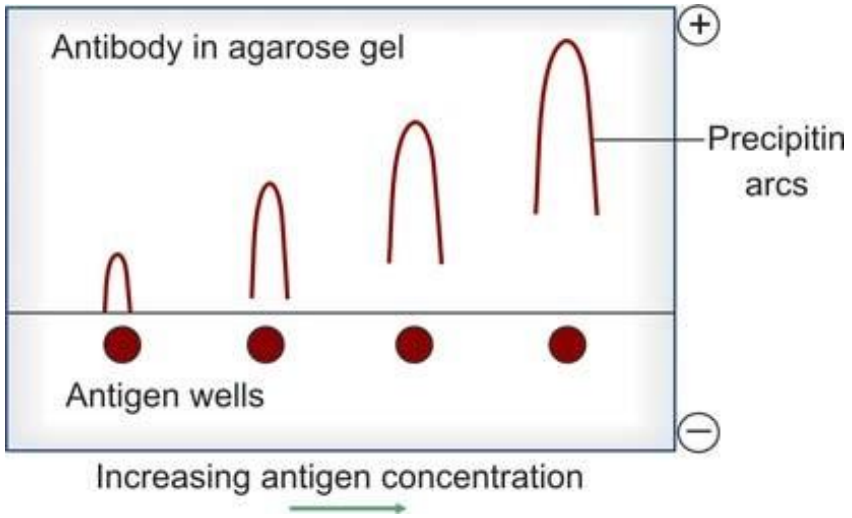



**Figure 7.10. Crossed immunoelectrophoresis**

The procedure is performed under the same conditions as with immunoelectrophoresis. The advantage of this method over simple immunodiffusion includes the speed with which results can be obtained (less than one hour), and the requirement of fewer antigens and antibodies and at least 10 times more sensitive than simple immunodiffusion. CIE was used to detect HBsAg, alpha-fetoprotein in blood serum and specific bacterial and cryptococcal antigen in cerebrospinal fluid in meningitis [5, 6, 7].

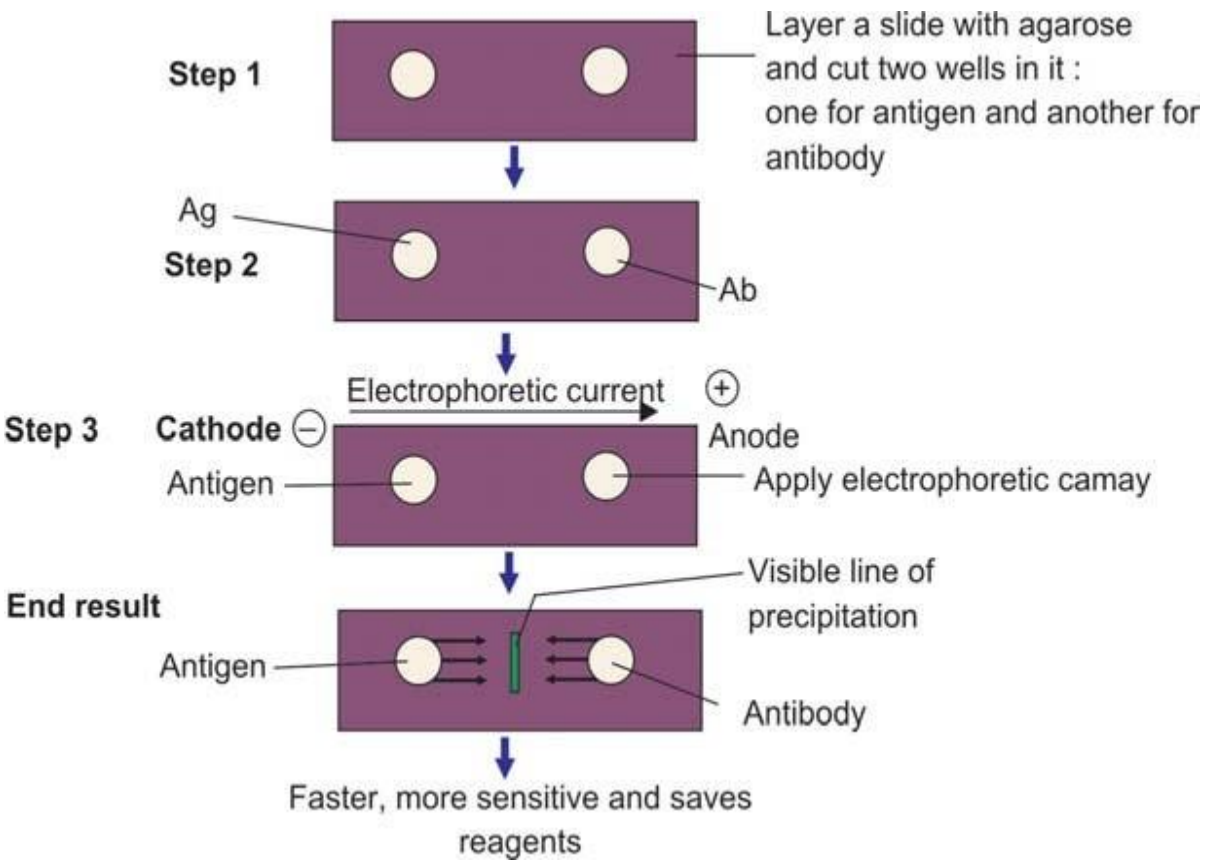



**Figure 7.11. Steps in counter current immunoelectrophoresis**

## AGGLUTINATION

Unlike precipitation, in an agglutination reaction, an antigen is a part of the surface of some dispersed material, such as an erythrocyte, a bacterium, or an inorganic particle, such as polystyrene latex, that has been coated with an antigen. An antibody added to a suspension of such particles combines with a surface antigen and binds them together to form a clearly visible aggregate, which is called agglutination.

### Principle

The principle of lattice formation, which explains deposition, is also applicable to agglutination. Agglutination is mainly, but not always, used to detect antibodies to which it is more sensitive.

**Factors Influencing Agglutination Reaction**

The agglutination reaction is facilitated by increased temperature and movements (for example, shaking, stirring, centrifugation, etc.), which increase the contact between the antigen and the antibody. Aggregation of clumps requires the presence of salts. Incomplete or monovalent antibodies do not cause agglutination, although they combine with an antigen. They can act as blocking antibodies by inhibiting agglutination with a complete antibody added afterwards.

**Applications of Agglutination Reaction**

**Tube agglutination.** The in vitro agglutination test for brucellosis may be complicated by the phenomenon of prozona and the presence of blocking antibodies. Therefore, several dilutions of serum should be tested to avoid false negative results due to prozone. Incomplete or blocking antibodies can be detected by performing a test in hypertonic saline solution or using a Coombs antiglobulin test.

This is done in tubes with a round bottom or plexiglass plates with round holes, and a series of double dilutions of antiserum is made in the tubes. After adding the antigen in the form of particles, the mixture is incubated. The last tube (or well) in which agglutination is clearly visible is the endpoint of the test, and the dilution of antiserum at the end point is called its titer. This is an indicator of the number of antibody units per unit volume of serum. In this type of tests, care should be taken to avoid the phenomenon of prozona.

The test tube agglutination test is usually used for:

- Serological diagnosis of typhoid fever,
- Brucellosis and typhus,
- The Felix Weil test is performed for typhus and
- Widal test for serological diagnosis of typhoid fever.

The *in vitro* agglutination test for brucellosis may be complicated by the phenomenon of prozona and the presence of blocking antibodies. Therefore, several dilutions of serum should be tested to avoid false negative results due to

prozone. Incomplete or blocking antibodies can be detected by performing a test in hypertonic saline solution or using a *Coombs antiglobulin test.*

The Coombs test was developed in 1945 by Coomb's, Mourant and Race to detect anti-Rh antibodies that do not agglutinate Rh-positive erythrocytes in saline. When mixing serums containing incomplete anti-Rh antibodies with Rh-positive erythrocytes, agglutination does not occur, since the surfaces of erythrocytes are covered with antibody globulin. For agglutination, rinse these red blood cells to get rid of unbound proteins, and then mix with rabbit antiserum (Coombs serum) against human gamma globulins (Figure 7.12.). The Coombs test can be of direct and indirect type. In the direct Coombs test, the sensitization of erythrocytes with incomplete antibodies occurs in vivo, as in hemolytic disease of newborns due to Rh incompatibility. In an indirect test, the sensitization of erythrocytes with antibody globulin is carried out in vitro.

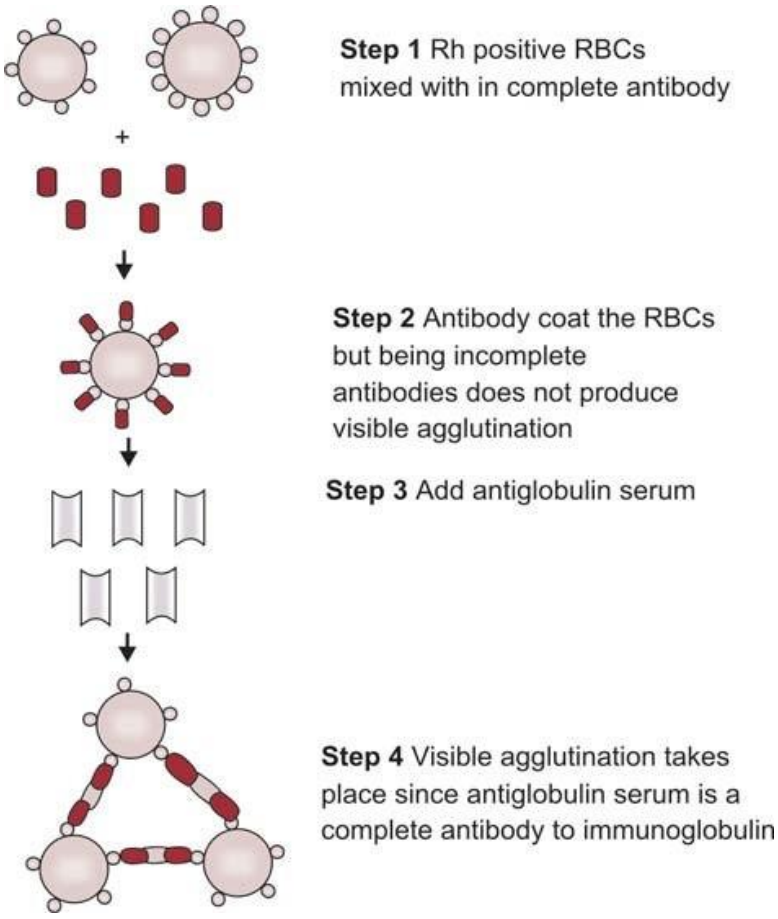



**Figure 7.12. Coomb's (Antiglobulin) test**

Sensitization of erythrocytes *in vivo* occurs with incomplete antibodies. This occurs with fetal erythroblastosis. When these cells are washed to remove unbound proteins and Coombs serum is added, agglutination occurs. In the indirect Coombs test, the sensitization of erythrocytes with antibody globulins is carried out *in vitro.* The Coombs test is also used to demonstrate incomplete antibodies in other diseases such as brucellosis.

**Slide agglutination.** This method is useful where only small number of culture are available; where agglutination is carried out with undiluted serum, and this is only applicable when agglutination occurs within a minute or so. Here are some of the important examples of using this technique:

• Detection of Bordetella whooping cough,

• Typing of pneumococci and streptococci

• Confirmation of the diagnosis for the presence of salmonella and shigella organisms.

• This method is also used for blood grouping and cross-matching.

The test can be easily carried out on a slide. A small amount of culture is added to a drop of saline solution and emulsified in it. With the help of a platinum loop, a drop of serum is placed on a slide next to the bacterial suspension. Both are mixed and explored. Agglutination, when it occurs, occurs quickly and can be visible to the naked eye, but using some form of magnification is an advantage. Appropriate controls should be installed on the slide.

Although the slide agglutination test is fast and convenient, it has limitations associated with random non-specificity, especially when high titer serum is used in undiluted form. Any ambiguity in the agglutination of the slide should be checked by in vitro agglutination [5, 6].

**Agglutination absorption tests.** When a serum comprising an antibody against a particular organism is mixed with a homologous organism and the mixture is centrifuged, it is seen that the antibody is ‘absorbed' or removed by organisms from the serum. This absorption is useful in the cultivation of antiserum

in animals. When an animal is immunized with a certain bacterium, ‘group antibodies’ to related organisms are produced. In some cases, they may be present in a high titer. Absorption by a heterologous strain will result in the removal of only group-specific antibodies, without affecting specific antibodies. These effects are illustrated in the Salmonella and Brucella groups.

**Coagglutination.** This method was entered in 1973 by Kronvall for serological typing of pneumococci. The test is based on the presence of protein A on the surface of some strains of Staphylococcus aureus (especially Cowan I), which can non-specifically bind any IgG molecule through its Fc part. Thus, part of the Fab remains free to bind to a specific antigen. Whenever Fab combines with a specific antigen, the reaction becomes visible due to the adhesion of *Staphylococcus aureus* (Figure 7.13).

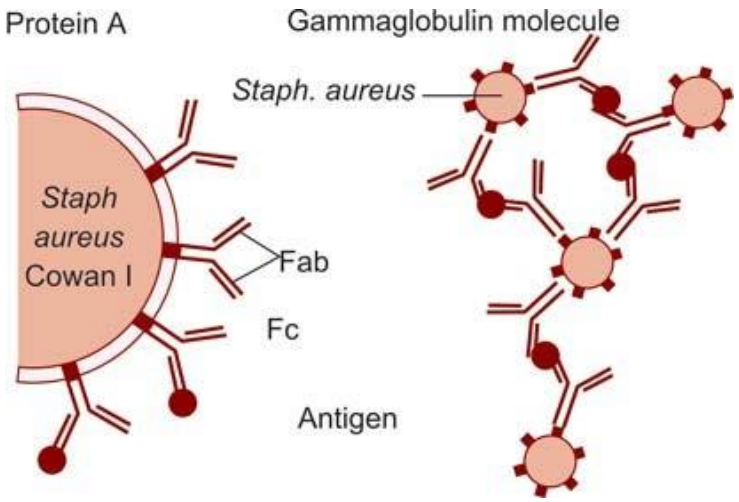



**Figure 7.13. Coagglutination**

The agglutination reaction is carried out in the form of a simple slide test with a suspension of a suspected homologous antigen prepared from a bacterial culture, or alternatively, the test can be used directly for detection:

- The presence of bacterial antigens in blood serum and urine.
- Commercial reagents are currently available for the identification of

*Neisseria gonorrhoeae* of the *Staphylococcus aureus* serogrouping.

- Reagents for detecting meningococcal, pneumococcal and hemophilic antigens in cerebrospinal fluid are similarly available. But the LIQUID must be absorbed by stabilized staphylococci to prevent nonspecific agglutination with human IgG [6, 8].

## COMPLEMENT FIXATION TEST

Complement is a normal component of serum and is enabled when it connect with the antigen-antibody complex. This connection has no visible effect. Therefore, it is necessary to use an indicator system consisting of sheep erythrocytes coated with an antibody against sheep erythrocytes. Complement has the property of lysing cells coated with antibodies.

In the test, the antibody, complement and antigen are blended, together and after the incubation period, an indicator system is added - antibody-coated sheep cells. However, the complement would be absorbed at the incubation stage by the initial antigen-antibody complex and would not be available for erythrocyte lysis. Thus, a positive complement fixation test indicates the absence of erythrocyte lysis, while a negative test with unused complement indicates erythrocyte lysis. (Figure 7.14.).

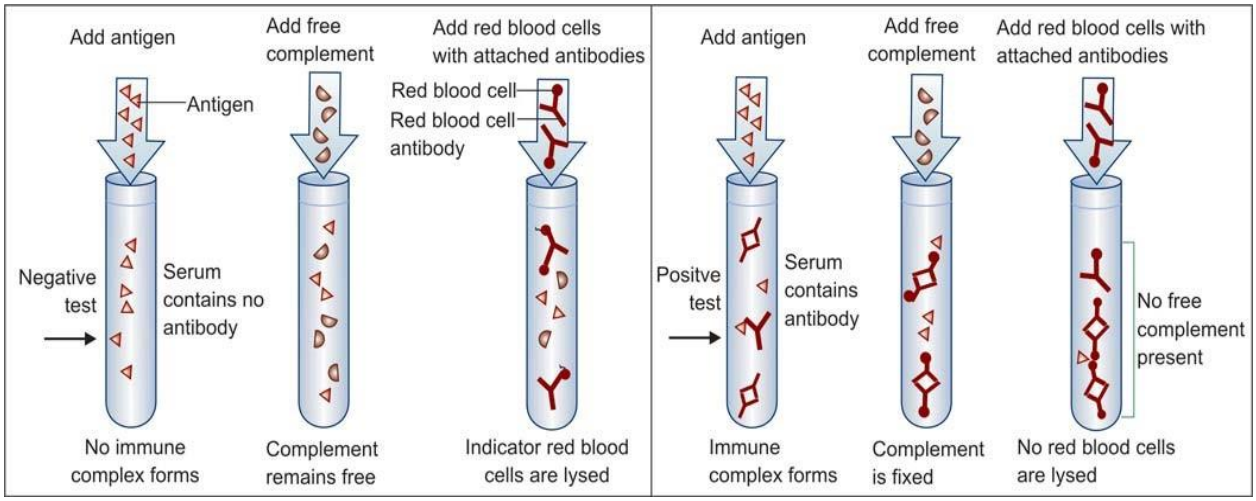


**Figure 7.14. Complement fixation test**

The antigen in this test can be soluble or dispersed. Before starting the test, the serum should be inactivated by heating it at 56 °C for 30 minutes to destroy any complement activity that the serum may have, as well as to remove some nonspecific complement inhibitors. The best source of complement for laboratory use is guinea pig serum [3, 7, 8].

## NEUTRALIZATION REACTIONS

These are basically of two types (Figure 7.15.): a) toxin neutralisation tests; b) virus neutralisation tests.

When neutralizing the toxin, homologous antibodies prevent the biological effect of the toxin observed in vivo in experimental animals (for example, intradermal administration of a mixture of Clostridium perfringens toxin and antitoxin to guinea pigs or mice) or using special nutrient media in vitro (for example, Nagler reaction and antistreptolysin O test).

In virus neutralization tests, different methods are available that can be used to establish the identity of the virus, as well as to evaluate antibodies against the virus. These tests can be carried out on animals, eggs, as well as on cell cultures [5].

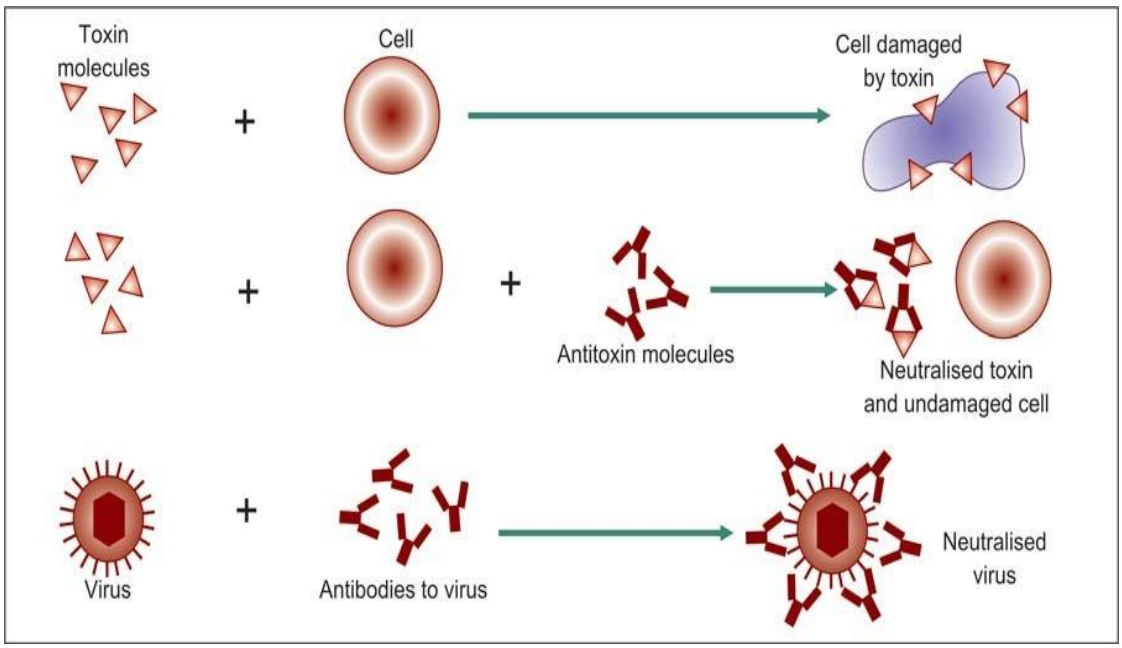



**Figure 7.15. Neutralization reactions**

## IMMUNOFLUORESCENCE

Fluorescent dyes are often used fluorescein isothiocyanate (FITC) and lissamine-rhodamine. These dyes exposition fluorescence by absorbing UV light in the range from 290 to 495 nm and releasing, colored light with a longer wavelength of 525 nm, which gives the protein labeled with the dye a radiant appearance (fluorescence). Blue-green fluorescence is observed when using fluorescein isothiocyanate, and when using rhodamine lissamine, it is orange-red.

This method is more sensitive than deposition or additional fixation methods. This method can easily identify at a concentration of about 1 μg of protein/ml of body fluid. The main disadvantage of this method is the frequent occurrence of nonspecific fluorescence in tissues and other materials.

There are two main methods used. ***The direct method*** consists in bringing fluorescein-labeled antibodies into contact with antigens fixed on a slide, involving them to react by washing out excess antibodies and examining under a fluorescent microscope. The compound of the labeled antibody with its antigen can be seen using apple-green fluorescence on a slide (Figure 7.16.).

***The indirect method*** can be used both to identify specific antibodies and to identify antigens. First, a layer of unmarked antiserum is applied. The reaction of this antiserum with the material on the slide is shown using a fluorescein-labeled antiglobulin serum specific to the antiserum globulin applied first. This test is more sensitive than the direct method. Suitable conjugates are widely available commercially. Similarly, the antigen-antibody complex can be detected by demonstrating the presence of a complement that attaches to the Ag-Ab complex using a FITC-conjugated antibody to supplement the C3 protein (in Figure 7.16.).

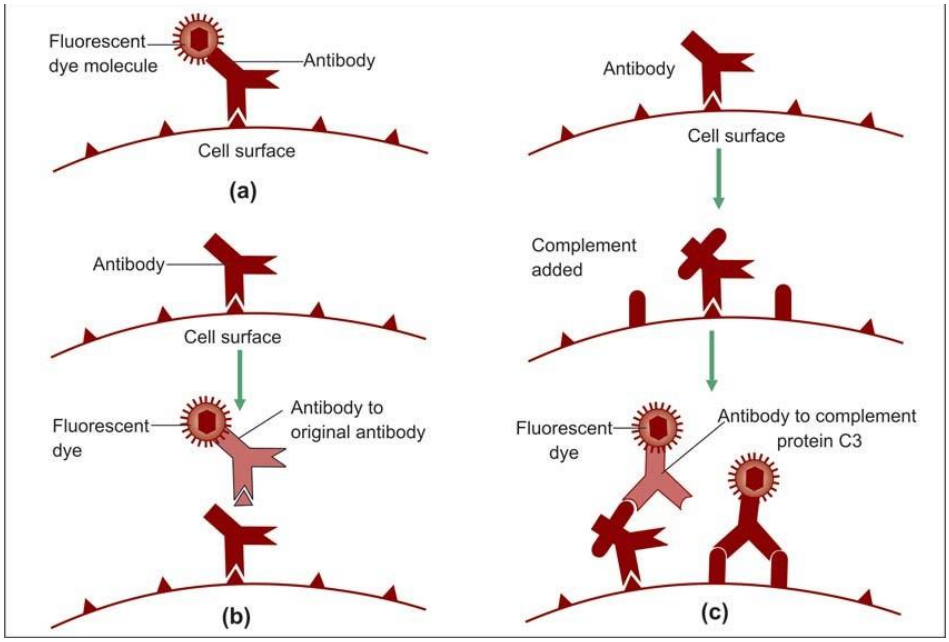



**Figure 7.16. Immunofluorescence**

Instead of marking with a fluorescent dye, antibodies marked with an enzyme were also used. Otherwise, the principle remains the same as with immunofluorescence. The commonly used enzyme is horseradish peroxidase. The advantage of this method is long-term storage, whereas fluorescein preparations fade after exposure to ultraviolet light [6, 8].

## ENZYME IMMUNOASSAYS

They are usually called enzyme linked immuno-sorbent assays or ELISA. This is a simple and diverse methods that is as sensitive as radioimmunological analyses. ELISA is perhaps currently the most widely used method for detecting antigens, antibodies, hormones, toxins and viruses.

The antibodies are conjugated with the enzyme by adding glutaraldehyde, so that the resulting antibody molecule has both immunological and enzymatic activity and is quantified by their capacity to decompose as a suitable substrate. Commonly used enzymes are alkaline phosphatase and horseradish peroxidase. Their relevant substrates are p-nitrophenyl phosphate and O-phenylenediamine dihydrochloride. The enzymatic activity leads to a color change that can be visually assessed or quantified using a simple spectrophotometer (Figure 7.17).

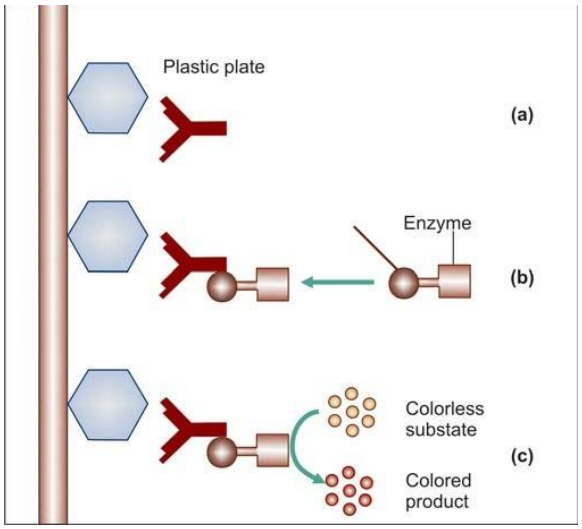



**Figure 7.17. ELISA**

The ELISA can be made with sensitized carrier surfaces in the form of polystyrene tubes (macro-ELISA) or polyvinyl plates for microtitration (micro-ELISA) or even balls.

Currently, several variants of ELISA methods are available to provide simple diagnostics for clinical and inpatient services. These include card and probe methods [5].

## RADIOIMMUNOASSAY

It is an extremely sensitive technique in which antibody or antigen is tagget with a radioactive material ($I^{125}$). The quantity of radioactive material in the antigen- antibody complex can be measured, with which the concentration of the antigen or antibody can be determined (Figure 7.18.). Radioimmunological analysis was first outlined in 1959 by Berson and Yalow. The changes made to the test help detect proteins in quantities up to a picogram.

This test is also named as binder-ligand assay, where the binder is the component to which the radioactive material is labeled, and the ligand (or analyte) is the component (antigen or antibody) to be analyzed or detected. In radioimmunological analysis, a fixed amount of an antibody and an antigen labeled with radioactive material react in the presence of an unlabeled antigen (the antigen being tested). After the reaction, the ‘free’ and ‘bound' fractions of the antigen are separated and their radioactivity is measured. The concentration of the tested antigen can be calculated by the ratio of the associated and total antigen label using appropriate standards [6].

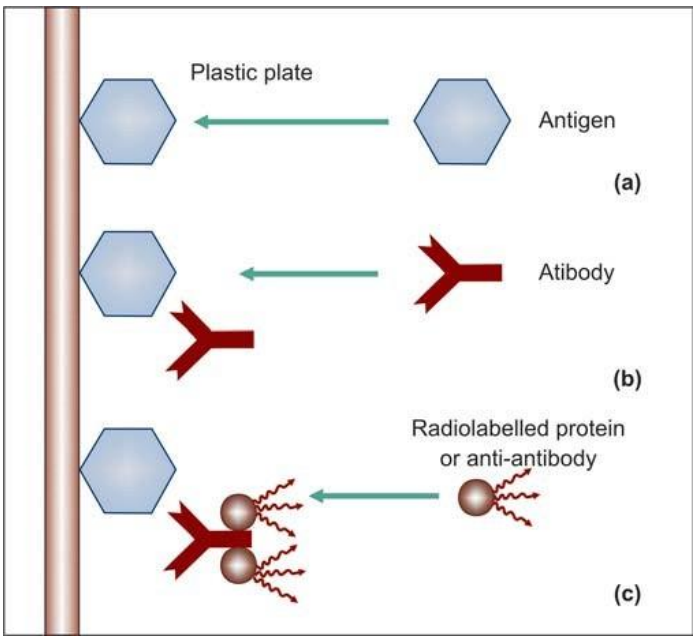



**Figure 7.18. Radioimmunoassay**

## MISCELLANEOUS TESTS

### Immune Electron Microscopy

The combination of antibodies with viral or surface antigens causes adhesion of viral particles, which can be visualized under an electron microscope. This method is mainly used to detect diarrhea and hepatitis viruses.

### Capsule Swelling Tests

When capsulated bacteria are blended with homologous antiserum, the complex makes easily visible under microscope. Pneumococcus and *Klebsiella* are two bacteria that cause this reaction, which was previously thought to be a suppression or swelling reaction.

### Immunoferritin Test

Ferritin is an electron-dense substance that is received from the spleen of a horse and can be conjugated with an antibody. This antibody is permitted to combine with the antigen, and the electron-dense nature of the label facilitates visualization under an electron microscope.

### Recognition of Immune Complexes

Currently, it is known that many diseases are caused by immune complexes.

Focal nephritis and endocarditis in streptococcal infection, as well as liver damage and polyarthritis in hepatitis B are some important examples. These immune complexes can be detected in various ways, which include cryopreservation, deposition with polyethylene glycol and detection of the complement component in immune complexes. Commercial kits are also available for the diagnosis of certain clinical conditions, such as bacterial endocarditis [6, 7, 8].

## OPSONIZATION

Opsonization is the process where particles such as microorganisms become covered with molecules which permit them to bind to receptors on phagocytes. Antibodies (especially IgG) and complement proteins like C3b can opsonize and are therefore called to as “opsonins”. An opsonin is any molecule that acts as a binding enhancer for the process of phagocytosis.

The IgG antibodies bind to the antigens with the Fab region outgoing the Fc region sticking out. Phagocytes have Fc gamma receptors and therefore they can bind to the covered molecules and absorb them. The complement fragment, C3b, nonspecifically binds to foreign organism. Phagocytes also have receptors for C3b, on their surface. The antibodies and C3b tag the microorganisms for destruction by phagocytes.

*Examples of opsonins are:*

- antibodies: IgG and IgA
- components of the complement system: C3b, C4b.

Antibody opsonization is when antibodies opsonize a causative agent, making it ready for splitting [2, 4].

**4. Illustrative material**: tables, charts, posters, multimedia projector.

**5. Literature:**

1. Atazhakhova M.G., IMMUNOLOGY:Training manual-Maykop: Publisher IB Kucherenko V.O., 2020. – 56 p

2. Rajesh Bhatia, Rattan Lal Ichhpujani - Essentials of Medical Microbiology: textbook for universities. – 4th edition, Jaypee Brothers Medical Publishers *Editorial Consultant:* Ms Peromila MA (English), 2008

3. Subhash Chandra Parija - Textbook of Microbiology and Immunology - 2nd Edition, Published by Elsevier, a division of Reed Elsevier India Private Limited: Elsevier, 2012.

4. Murphy K.M, Travers P, Walport M. Janeway's immunobiology. 7th ed. New York: Garland Science; 2007.

5. N. Parker, M. Schneegurt, Anh-Hue Thi Tu, B.M. Forster, P. Lister, - Microbiology, textbook, - Houston, Texas: 2017 Rice University, - 4.0 International License (CC BY 4.0).

6. Medical Microbiology, Virology and immunology; M42 Textbook for medical students / Ed. edited by A. A. Vorobyov. — 2nd ed. Rev. and extra— M.: OOO "Medical information Agency", 2012. — 704 S.: ill., table. ISBN 978-5-8948-1895-5

7. A. A. Yarilin "Immunology", textbook, Moscow, publishing group "GEOTAR-Media", 2010.

8. Kazmirchuk V. E., Koval'chuk L .In. Maltsev D. V. K14 Clinical immunology and Allergology./ Kazmirchuk V. E., Kovalchuk JI.B., Maltsev D. V. — K.: Phoenix, 2009. — 524 p. — (In Russian). ISBN 978-966-651-730-5

**6. Checklist:**

1. Serological reactions, their practical application in medicine.
2. Group serological reactions.
3. Agglutination.
4. Precipitation
5. Applications of Precipitation reactions
6. Neutralization reactions
7. Immunofluorescence
8. Miscellaneous tests

## LECTURE № 8.

### 1. TOPIC: THE CONCEPT OF ALLERGENS

**2. Purpose:** To familiarize students with the main mechanisms of hypersensitivity according to the Coombs and Gell classification, to consider the pathogenesis and clinical examples of the main types of hypersensitivity.

**3. Abstracts of the lecture**

**Allergy** is an altered reactivity of the body, which manifests itself in the disturbance of the usual course of general or local reactions, often during repeated entrance into the body of substances known as *allergens*. For indication of hypersensitivity reactions, the host should have had contact with the antigen (allergen). The initial contact sensitizes the immune system, leading to the priming of the appropriate B and T lymphocytes. This is aware as the "*sensitizing*" dose. Subsequent contact with the allergen causes increased of hypersensitivity. This is aware as the "*shocking*" dose.

- Type I (anaphylaxia, atopy) is mediated by **IgE**
- Type II (cytotoxic reaction) is mediated by **IgG** and **IgM**
- Type III (Immune complex hypersensitivity) is mediated by **IgG** and **IgM**
- Type IV (delayed hypersensitivity) is cell-mediated (mediated by **T-lymphocytes**)
- Type V (stimulatory hypersensitivity) [1].

Depending on the time taken for the reactions and the mechanisms that cause the tissue damage, hypersensitivity has been broadly classified into immediate type and delayed type. In the former, the response is seen within minutes or hours after exposure to the antigen and in the latter, the process takes days together to manifest as symptoms.

Prince of Monaco first observed the deleterious effects of jellyfish on bathers. Subsequently, Portier and Richet (1906) suggested a toxin to be responsible for these effects and coined the term "anaphylaxis".

Gell and Coombs (1963) classified hypersensitivity reactions into four categories based on the time elapsed from exposure of antigen to the reaction and the arm of immune system involved. Types I, II, and III are antibody-mediated and are known as ***immediate hypersensitivity*** reactions, while type IV is cell-mediated (i.e., mediated by cell-mediated immunity) and is known as ***delayed hypersensitivity*** reactions.

Type V hypersensitivity reaction has been described later. It is called stimulatory type reaction and is a modification of type II hypersensitivity reaction.

Distinguish between immediate and delayed hypersensitivities have been summarized in Table 8-1 [1, 2].

**Table 8-1. Distinguish between immediate and delayed hypersensitivities**

| Properties Immediate Delayed | Properties Immediate Delayed | Properties Immediate Delayed |
|---|---|---|
| **Type** | I, II, III | IV |
| **Time to manifest** | Minutes to hours | Days |
| **Mediators** | Antibodies | T cells |
| **Route of sensitization** | Any route | Intradermal |
| **Passive transfer with serum** | Possible | Not possible |
| **Desensitization** | Easy but short lived | Difficult but long lasting |

## TYPE I (ANAPHYLACTIC) HYPERSENSITIVITY

Type I hypersensitivity reaction is commonly called allergic or immediate hypersensitivity reaction. This reaction is always rapid, occurring within minutes of exposure to an antigen, and always involves IgE-mediated degranulation of basophils or mast cells.

Type I reactions are also known as IgE-mediated hypersensitivity reactions. IgE is responsible for sensitizing mast cells and providing recognition of antigen for immediate hypersensitivity reactions. The short time lag between exposure to antigen and onset of clinical symptoms is due to the presence of preformed

mediators in the mast cells. Thus, the time taken for these reactions to initiate is minimal, so the onset of symptoms seems to be immediate. Type I reaction can occur in two forms: anaphylaxis and atopy [3].

**Anaphylaxis**

Anaphylaxis is an acute, potentially fatal, and systemic manifestation of immediate hypersensitivity reaction. This occurs when an antigen (allergen) binds to IgE on the surface of mast cells, followed by the release of several anaphylaxis mediators. On exposure to the antigen, TH2 cells specific to the antigen are activated, leading to the stimulation of B cells to produce IgE antibody (Figure 8-1). The IgE then binds to Fc portion of mast cells and basophils with high affinity. On reexposure to the antigen, the allergen crosslinks the bound IgE, followed by activation of IgE and degranulation of basophils and mast cells to release pharmacologically active mediators within a few minutes.

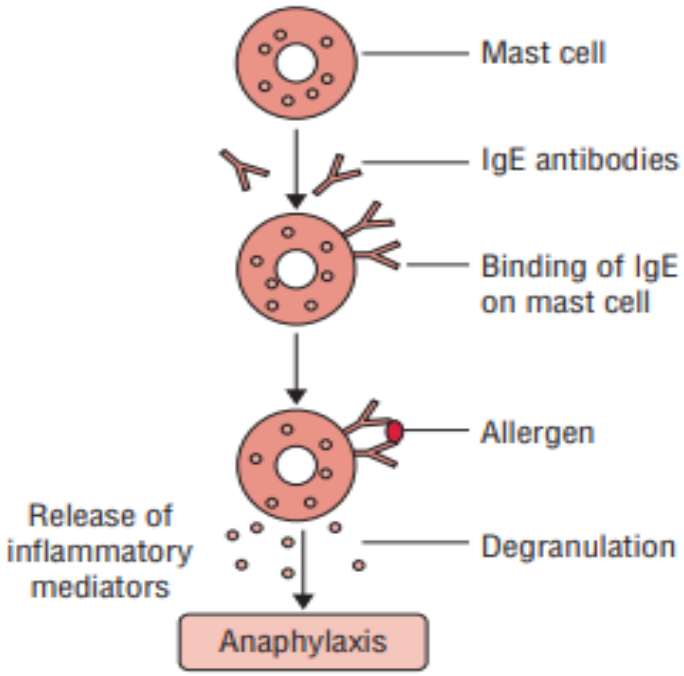



**Figure 8.1. A schematic diagram showing type I hypersensitivity reaction.**

Binding of IgE to the mast cells is also known as sensitization, because IgE-coated mast cells are ready to be activated on repeat antigen encounter.

**Initiator cells in anaphylaxis**

The initiator of type I reaction is otherwise known as allergen. Typical allergens include:

- Plant pollen, proteins (e.g., foreign serum and vaccines),
- Certain food items (e.g., eggs, milk, seafood, and nuts),
- Drugs (e.g., penicillin and local anesthetics),
- Insect products (venom from bees, wasps, and ants),
- Dust mites, mould spores, and
- Animal hair and dander.

The precise reason for these substances to act as allergens is not known, although they show some common characteristics. Because these reactions are T-cell dependent, T-cell-independent antigens like polysaccharides cannot elicit type I reactions.

**Effector cells in anaphylaxis**

The effector cells in anaphylaxis include (a) mast cells, (b) basophils, and (c) eosinophils. All these three cells contain cytoplasmic granules whose contents are the major mediators of allergic reactions. Also, all these three cell types produce lipid mediators and cytokines that induce inflammation.

**Mast cells:** Mast cells are the prime mediators of anaphylaxis. These cells are found throughout connective tissue, particularly near blood and lymphatic vessels. IgE-mediated degranulation of mast cells occurs when an allergen causes cross-linkage of the membrane-bound IgE. The importance of cross-linkage in the process can be understood by the fact that monovalent molecules, which cannot cause cross-linkage, are unable to cause degranulation.

**Mediators of anaphylaxis**

Many substances instead of a single substance are responsible for all manifestations of anaphylaxis. Important mediators include (a) histamine, (b) slow-reacting substances of anaphylaxis (SRS-A), (c) serotonin, (d) eosinophilic chemotactic factors of anaphylaxis, and (e) prostaglandins and thromboxanes. Histamine: This is the most important mediator of anaphylaxis. It is found in a

preformed state in the granules of mast cells and basophils. This causes vasodilatation, enlarged capillary permeability and contraction of smooth muscle.

It is the main mediator of allergic rhinitis (hay fever), urticarial and angioedema. Antihistamines that block histamine receptors are relatively effective against allergic rhinitis, but not against asthma [2].

**Slow-reacting substances of anaphylaxis:** These are produced by leukocytes. They consist of several leukotrienes that do not occur in a preformed state, but are formed during anaphylaxis reactions.

Leukotrienes are principal mediators of bronchoconstriction in asthma and are not inhibited by antihistamines. They cause enlarged vascular permeability and contraction of smooth muscle.

**Serotonin**: Serotonin is found in preformed state in mast cells and platelets. This causes vasoconstriction, enlarged capillary permeability and contraction of smooth muscle.

**Eosinophilic chemotactic factors of anaphylaxis:** It is found in preformed state in granules of mast cells. It attracts eosinophils to the site of action. The role of eosinophils, however, is not clear in type I hypersensitivity reaction. Nevertheless, it is believed to reduce severity of type I hypersensitivity by releasing the enzymes histaminase and arylsulfatase that degrade histamine and SRS-A, respectively.

**Prostaglandins and thromboxanes:** Prostaglandins cause bronchoconstriction as well as expansion and increased permittivity of capillaries. Thromboxanes cause aggregation of platelets.

All these mediators are very quickly inactivated by enzymatic reactions, therefore, they are active only for a few minutes after their release.

**Phases of anaphylaxis**

The spectrum of changes seen in type I hypersensitivity can be considered under immediate and late phases.

**Immediate phase:** This phase is characterized by degranulation and release of pharmacologically active mediators within a few minutes after repeated exposure to the same antigen.

Histamine is the principal biogenic amine that causes rapid vascular and smooth muscle reactions, such as vascular leakage, vasodilatation, and bronchoconstriction. It is responsible for the "wheal and flare" response seen in cutaneous anaphylaxis and also for the increased peristalsis and bronchospasm associated with ingested allergens and asthma, respectively.

**Late phase:** This phase begins to develop 4–6 hours after the immediate phase reaction and persists for 1–2 days. It is characterized by the infiltration of neutrophils, macrophages eosinophils, and lymphocytes to the site of reaction. This leads to an amplification of the various inflammatory symptoms seen as a part of the early reaction like bronchoconstriction and vasodilatation. The cells remain viable after degranulation and proceed to synthesize other substances that are released at a later time, causing the late phase of type I reactions. The mediators are not detectable until after some hours of the immediate reaction. The important mediators involved during the late phase are:

■ slow-reacting anaphylaxis substances (SRS-A) that contain several leukotrienes (e.g., LTC4, LTD4, and LTE4);

■ platelet-aggregating factor; and

■ cytokines released from the mast cells [1, 4].

**Clinical manifestations of anaphylaxis**

Anaphylaxis is an acute, life-threatening reaction usually affecting multiple organs. The time of onset of symptoms according to the level of hypersensitivity and the amount, diffusivity, and localization of exposure to the antigen.

Multiple organ systems are usually affected, including the skin (pruritus, flushing, urticaria, and angioedema), respiratory tract (bronchospasm and laryngeal edema), and cardiovascular system (hypotension and cardiac arrhythmias). When death occurs, it is usually due to laryngeal edema, intractable bronchospasm,

hypotensive shock, or cardiac arrhythmias developing within the first 2 hours. **Anaphylactoid reaction:** This appears to be clinically similar to anaphylactic reaction but differs from it in many ways. Firstly, it is not mediated by IgE. Secondly, provoking agents (such as madication or iodine-containing agents) directly stimulate basophils and mast cells to release mediators without any involvement of the IgE.

**Management and prevention of anaphylaxis**

Desensitization is an effective way for prevention of systemic anaphylaxis. It is of two types: acute desensitization and chronic desensitization.

***Acute desensitization*** involves the administration a lot of an antigen to which the person is sensitive, at an interval of 15 minutes. The complex of antigen–IgE is produce small quantities; hence enough mediators are not released to produce a major reaction. However, this action is short lived because of the return of hypersensitivity reaction due to continued production of IgE.

***Chronic desensitization*** involves the long-term administration of antigen to which the person is sensitive, at an interval of weeks. This stimulates the production of IgA- and IgG-blocking antibodies that prevent subsequent antigen to binding to mast cells, therefore, preventing the reaction.

**Atopy**

The term atopy was first coined by Coca (1923) to denote a condition of familial hypersensitivities that occur spontaneously in humans. Atopy is recurrent, nonfatal, and local manifestation of immediate hypersensitivity reaction.

The reaction shows a high degree of familial predisposition and is associated with a high level of IgE. It is localized to a specific tissue, often involving epithelial surfaces at the site of antigen entry. It is mediated by IgE antibodies, which are homocytotropic (i.e., species specific). Only human IgE can fix to surface of mast cells.

Common manifestations of atopy are asthma, rhinitis, urticaria, and atopic dermatitis. The commonest of atopic reactions is bronchial asthma [3, 5].

## TYPE II (CYTOTOXIC) HYPERSENSITIVITY

Type II cytotoxic reaction is mediated by antibodies directed against antigens on the cell membrane that activates complement thereby causing antibody-mediated destruction of cells (Figure 8.2). The cell membrane is damaged by a membrane attack complex during activation of the complement. The reactions involve combination of IgG or IgM antibodies with the cell-fixed antigens or alternately circulating antigens absorbed onto cells. Antigen–antibody reaction leads to complement activation, leading to the formation of a membrane attack complex. This complex then acts on the cells, causing damage to the cells, as seen in complement-mediated lysis in Rh hemolytic disease, transfusion reaction, or hemolytic anemia. Similarly, the antibodies combining with tissue antigen contribute to the pathogenesis of Goodpasture's syndrome, pemphigus, and myasthenia gravis. Antibody-dependent cell-mediated cytotoxicity (ADCC): It is another mechanism, which involves the binding of cytotoxic cells with Fc receptors in the Fc binding part of the antibodies coating the target cells. The antibody coating the target cell can also cause its destruction by acting as an opsonin. This mechanism is important in immunity against large-sized pathogens, such as the helminths [3].

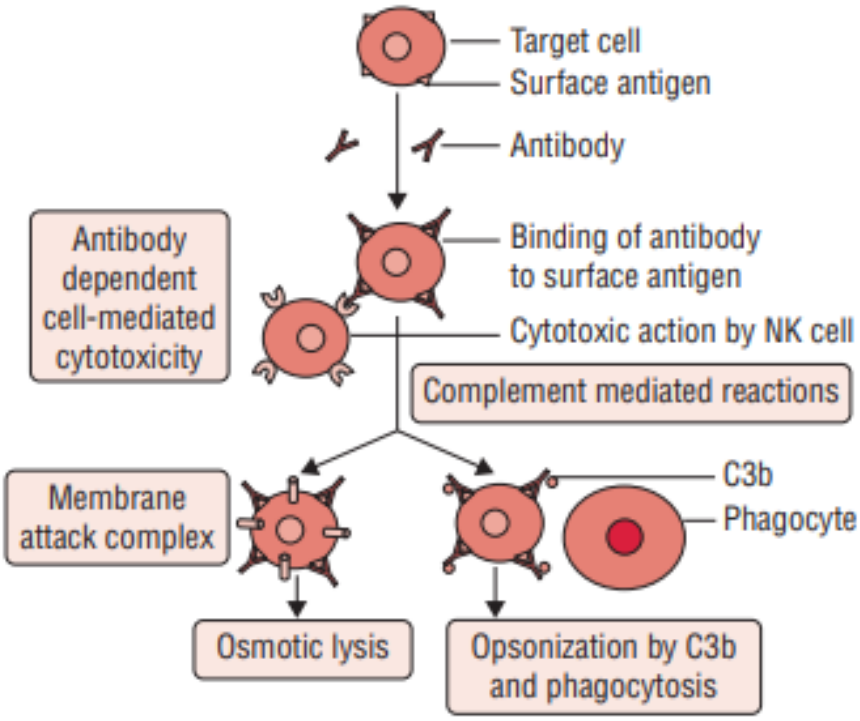



**Figure 8.2. A schematic diagram showing type II hypersensitivity reaction.**

### Transfusion Reactions

A large number of proteins and glycoproteins are present on the surface of RBCs, of which A, B, and O antigens are of particular importance. Antibodies to these antigens are called isohemagglutinins and are of IgM class. When transfusion with mismatched blood occurs, a transfusion reaction takes place due to the destruction of the donor RBCs through the isohemagglutinins against the foreign antigen.

The clinical manifestations result from the massive intravascular hemolysis of the donor cells by antibody and complement.

### Erythroblastosis Fetalis

This condition develops when maternal antibodies specific for fetal blood group antigens cross the placenta and destroy fetal RBCs. This condition is observed in cases when a presensitive Rh-negative mother mounts an immune response against Rh-positive RBCs of the fetus. This results in severe hemolysis, leading to anemia and hyperbilirubinemia, which can even be fatal.

### Drug-Induced Hemolysis

Certain drugs (such as penicillin, quinidine, phenacetin, etc.) may induce hemolysis of red blood cells. They attach to the surface of red blood cells and induce formation of IgG antibodies. These autoantibodies then react with red blood cell surface, causing hemolysis. Similarly, quinacrine attaches to the platelet surface and evoke autoantibodies that lyse platelets, causing thrombocytopenia.

### Goodpasture's Syndrome

Autoantibodies of IgG class are produced against basement membrane of the lungs and kidneys in Goodpasture's syndrome. Such autoantibodies bind to tissues of the lungs and kidneys and activate the complement that leads to an increased production of C5a, a component of the complement. The C5a causes attraction of leukocytes, which produce enzyme proteases that act on lung and kidney tissues, causing damage of those tissues.

### Rheumatic Fever

In this condition, antibodies are produced against group A streptococci that cross-react with cardiac tissues and activate complement and release of components of complement, which in turn causes damage of cardiac tissues [1, 3, 5].

## TYPE III (IMMUNE COMPLEX) HYPERSENSITIVITY

Type III reaction is mediated by antigen–antibody immune complexes, which induce an inflammatory reaction in tissues.

### Mechanism of Immune-Complex Hypersensitivity

In many situations, reactions between the various antigens and antibodies in the body give rise to formation of immune complexes (Figure 8.3.). In the normal course, these immune complexes are normally removed by mononuclear-phagocyte system through participation of RBC. However, the body may be exposed to an excess of antigen in many conditions, such as persistent infection with a microbial organism, autoimmunity to self-components, and repeated contact

with environmental agents. When the clearance capacity of this system is exceeded, deposition of the complexes takes place in various tissues.

Immune complexes are deposited (a) on the walls of blood vessels, (b) in the synovial membrane of joints, (c) on the glomerular basement membrane of the kidneys, and (d) on the vascular plexus of the brain. Sometimes, immune complexes are formed at the site of inflammation itself. These in situ immune complexes, in certain cases, may be beyond the reach of phagocytic clearance and hence aggregate and cause disease.

Immune complexes fix complement and are potent activators of the complement system. Activation of the complement results in the formation of complement components, such as C3a- and C5a-anaphylatoxins that stimulate release of vaso-active amines. The C5a attracts neutrophils to the site, but these neutrophils fail to phagocytose large aggregated mass of immune complexes, and instead release lysosomal enzymes and lytic substances that damage host tissue.

The proteolytic enzymes (including neutral proteinases and collagenase), kinin-forming enzymes, polycationic proteins, and reactive oxygen and nitrogen intermediates cause damage in the local tissues and enhance the inflammatory responses. Platelets aggregated by intravascular complexes provide yet another source of vasoactive amines and may also form microthrombi, which can lead to local ischemia [3].

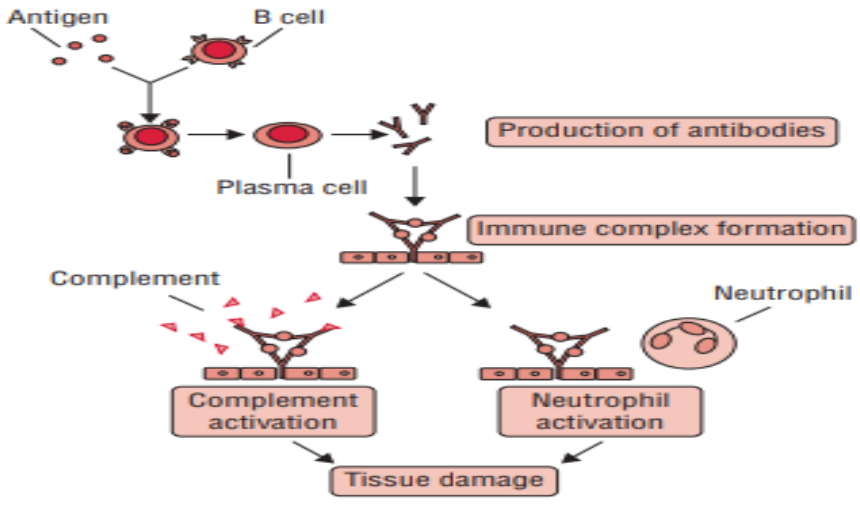



**Figure 8.3. A schematic diagram showing type III hypersensitivity reaction**

**Manifestations of Immune-Complex Hypersensitivity**

Arthus reactions and serum sickness reactions are two typical manifestations of type III hypersensitivity.

**Arthus reactions**

Arthus reaction is an inflammatory reaction caused by the deposition of immune complexes at a localized site. This reaction is named after Dr. Arthus who first described this reaction. This reaction is edematous in the early stages, but later can become hemorrhagic and, eventually, necrotic.

The lag time between antigen challenge and the reaction is usually 6 hours. This is considerably longer than the lag time of an immediate hypersensitivity reaction, but shorter than that of a delayed hypersensitivity reaction. Tissue damage is caused by deposition of antigen–antibody immune complexes and complement. The activation of complement through its product of activation causes vascular occlusion and necrosis.

**Serum sickness**

Serum sickness is a systemic inflammatory reaction caused by deposition of immune complexes at many parts of the body. The condition manifests after a single injection of a high concentration of foreign serum. It appears a few days to 2 weeks after injection of foreign serum or some medications, such as penicillin. However, serum sickness is considered as an immediate hypersensitivity reaction, because symptoms appear immediately after formation of immune complex.

Unlike type I hypersensitivity reaction, a single injection acts as both priming and shocking doses. Fever, lymphadenopathy, rashes, arthritis, splenomegaly, and eosinophilia are the typical manifestations. Disease is self-limited and clears without sequelae.

**Immune-Complex Diseases**

Formation of circulating immune complexes contributes to the pathogenesis of a number of conditions other than serum sickness. These include the following:

1. Autoimmune diseases

■ Systemic lupus erythematosus (SLE)

■ Rheumatoid arthritis

2. Drug reactions

■ Allergies to penicillin and sulfonamides

3. Infectious diseases

■ Poststreptococcal glomerulonephritis

■ Meningitis

■ Hepatitis

■ Infectious mononucleosis

■ Malaria

■ Trypanosomiasis [3, 5].

## TYPE IV DELAYED (CELL-MEDIATED) HYPERSENSITIVITY

Type IV hypersensitivity reaction is called delayed type hypersensitivity (DTH) because the reaction is delayed. This begins a few hours or days after initial contact with the antigen and often lasts for several days. The reaction is characterized by large influxes of nonspecific inflammatory cells, in particular, macrophages. It differs from the other types of hypersensitivity by being mediated through cell-mediated immunity. This reaction occurs due to the activation of specifically sensitized T lymphocytes rather than the antibodies.

Initially described by Robert Koch in tuberculosis as a localized reaction, this form of hypersensitivity was known as tuberculin reaction. Later, on realization that the reaction can be elicited in various pathologic conditions, it was renamed as delayed type hypersensitivity.

**Mechanism of DTH**

The DTH response begins with an initial sensitization phase of 1–2 weeks after primary contact with an antigen (Figure 8.4.):

■ TH1 subtypes CD4 are the cells activated during the sensitization phase.

■ A variety of antigen-presenting cells (APCs) including Langerhans cells and macrophages have been shown to be involved in the activation of a DTH response. These cells are believed to pick up the antigen that enters through the skin and transport it to regional lymph nodes, where T cells are activated by the antigen.

■ The APCs present antigens complexed in the groove of major histocompatibility complex (MHC) molecules expressed on the cell surface of the APCs.

■ For most protein antigens or haptens associated with skin DTH, CD4* T cells are presented with antigens bound to MHC class II alleles, human leukocyte antigen (HLA)-DR, HLA-DP, and HLA-DQ. Specific MHC class II alleles are recognized to produce excessive immune activation to antigens.

■ On subsequent exposure, the effector phase is stimulated. The TH1 cells are responsible in secreting a variety of cytokines that recruit and activate macrophages and other nonspecific inflammatory cells.

■ The response is marked only after 2–3 days of the second exposure. Generally, the pathogen is cleared rapidly with little tissue damage. However, in some cases, especially if the antigen is not easily cleared, a prolonged DTH response can itself become destructive to the host, as the intense inflammatory response develops into a visible granulomatous reaction [1].

**Types of DTH Reactions**

DTH reactions are of two types: contact hypersensitivity and tuberculin-type hypersensitivity reactions.

**Contact hypersensitivity**

Contact hypersensitivity is a manifestation of DTH occurring after sensitization with certain substances. These include drugs, such as sulfonamides and neomycin; plant products, such as poison ivy and poison oak; chemicals, such as formaldehyde and nickel; and cosmetics, soaps and other substances.

This reaction manifests when these substances acting as haptens enter the skin and combine with body proteins to become complete antigens to which a person becomes sensitized. On second exposure to the same antigen, the immune system responds by attack of cytotoxic T cells that cause damage, mostly in the skin.

The condition manifests as itching, erythema, vesicle, eczema, or necrosis of skin within 12–48 hours of the second exposure.

**Tuberculin-type hypersensitivity reaction**

The tuberculin reaction is a typical example of deferred hypersensitivity to antigens of microorganisms, which used for diagnosis of the disease.

**Tuberculin skin test:** This test is carried out to determine whether an individual has been exposed previously to *Mycobacterium tuberculosis* or not. In this test, a small amount of tuberculin (PPD), a protein derived from the cell wall of *M. tuberculosis*, is injected intradermally. Development of a red, slightly swollen, firm lesion at the site of injection after 48–72 hours indicates a positive test. A positive test indicates that the person was infected with the bacteria but does not show the presence of the disease, tuberculosis. However, if a person with a tuberculin-negative skin test becomes positive, then it indicates that the patient has been recently infected. The skin test, however, can even become negative in:

■ Infected persons receiving therapy with immunosuppressive drugs (such as corticosteroids and anticancer drugs) and

■ In those suffering from the diseases associated with suppressed cell-mediated immunity (such as AIDS, sarcoidosis, lymphoma, post measles vaccination, etc.).

The response to M. tuberculosis illustrates that while on one hand mechanisms involved in DTH are required for defense against the organism; on the other hand, these are also responsible for tissue damage in the longer run. Cytokines (like TNF and IFN-γ), which have been produced to activate the macrophages and thus contain the infection, also trigger other cascades that lead finally to extensive tissue damage.

Various other skin tests are used to detect DTH. These include many skin tests in bacterial, fungal, viral, and helminthic infections.

Lepromin test is a useful test for leprosy. A positive lepromin test indicates the presence of tuberculoid leprosy with intact cell-mediated immunity. On the other hand, a negative lepromin test indicates the presence of lepromatous leprosy with impaired cell-mediated immunity.

Positive skin tests in coccidioidomycosis, paracoccidioidomycosis and other fungal infections suggest exposure to the fungi. In both viral and parasitic infections, skin tests are less specific and less useful than the serological tests for diagnosis [3].

Differences between contact hypersensitivity and tuberculin-type hypersensitivity reaction are summarized in Table 8-2.

**Table 8-2 Distinguish between contact and tuberculin-type hypersensitivity**

| Characteristic | Contact hypersensitivity | Tuberculin-type hypersensitivity |
|---|---|---|
| Site | Epidermal | Intradermal |
| Antigen | Organic chemicals, poison ivy, metals, etc. | Tuberculin, lepromin, leishmanin skin tests, etc. |
| Reaction time | 48–72 hours | 48–72 hours |

## TYPE V (STIMULATORY TYPE) HYPERSENSITIVITY

In this type of hypersensitivity reaction, antibodies connect with antigens on cell surface, which encourages cells to proliferate and differentiate and enhances the activity of effector cells. Type V hypersensitivity reaction plays major role in pathogenesis of Graves' disease, in which thyroid hormones are produced in excess quantity. It is postulated that long-acting thyroid-stimulating antibody, which is an autoantibody to thyroid membrane antigen, combines with thyroid-stimulating hormone (TSH) receptors on a thyroid cell surface. Interaction with

TSH receptor produces an effect similar to the TSH, resulting in an excess production and secretion of thyroid hormone, which is responsible for Graves' disease.

Table 8-3 summarizes important features of different types of hypersensitivity [1,3,4].

**Table 8-3 Comparison of various types of hypersensitivity reactions**

| | **Type I** | **Type II** | **Type III** | **Type IV** |
|---|---|---|---|---|
| **Antigen** | Exogenous | Cell surface | Soluble | Tissue and organ |
| **Antibody** | IgE | IgG, IgM | IgG, IgM | None |
| **Reaction time** | 15–30 minutes | Minutes to hours | 3–8 hours | 48–72 hours |
| **Transfer** | Antibody | Antibody | Antibody | T cells |
| **Conditions** | Hay fever, allergy, and asthma | Erythroblastosis fetalis and Goodpasture's syndrome | SLE, serum sickness | Tuberculin test, poison ivy, etc. |

**4. Illustrative material**: tables, charts, posters, multimedia projector.

**5. Literature:**

1. Atazhakhova M.G., IMMUNOLOGY:Training manual- Maykop: Publisher IB Kucherenko V.O., 2020. – 56 p

2. Rajesh Bhatia, Rattan Lal Ichhpujani - Essentials of Medical Microbiology: textbook for universities. – 4th edition, Jaypee Brothers Medical Publishers *Editorial Consultant:* Ms Peromila MA/English, 2008

3. Subhash Chandra Parija - Textbook of Microbiology and Immunology - 2nd Edition, Published by Elsevier, a division of Reed Elsevier India Private Limited: Elsevier, 2012.

4. Murphy K.M, Travers P, Walport M. Janeway's immunobiology. 7th ed. New York: Garland Science; 2007.

5. N. Parker, M. Schneegurt, Anh-Hue Thi Tu, B.M. Forster, P. Lister, - Microbiology, textbook, - Houston, Texas: 2017 Rice University, - 4.0 International License (CC BY 4.0).

**6. Checklist:**

1. What is the allergy?

2.Which class of immunoglobulins plays a leading role in the pathogenesis of atopy?

3. Name the target cells in atopic allergic reactions

4. When administering which drugs can anaphylactic shock occur?

5. Which cells are engaged in the pathogenesis of GHT?

6. Tuberculin-type hypersensitivity reaction.

7. Mechanism of Immune-Complex Hypersensitivity.

8. How subdivided the antibody synthesis?

9. How many types has hypersensitivity?

10. Type V (stimulatory type) hypersensitivity.