



**AZERBAIJAN MEDICAL UNIVERSITY**  
**DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY**

**Lesson 17.**

**Immunity, its types: innate (non-specific) and acquired (specific). Innate (non-specific) immunity, its features and factors. Phagocytosis. Determination of phagocytic activity of leukocytes**

**FACULTY: General Medicine**  
**SUBJECT: Medical microbiology - 1**

## Discussed questions:

1. The concept, types and forms of immunity: innate and acquired.
2. Non-specific protective factors of the organism
  - non-specialized protective factors (skin and mucous membranes, normal microflora).
  - non-specific cellular factors (phagocytes, natural killers, dendritic cells, eosinophils, barrier cells).
  - non-specific humoral factors (complement, lysozyme, transferrin, C-reactive protein, quinine, cytokines: tumor necrosis factors, interferon).
3. Phagocytosis and its stages: complete and incomplete phagocytosis.
4. Opsonization phenomenon.
5. The killing of microorganisms in phagocytes: oxygen-dependent and oxygen-independent mechanisms.
6. Processing and its mechanism.
7. Determination of phagocytic activity (phagocytic index, phagocytic unit, opsono-phagocytic index, opsonic index, killing ability of phagocytes).

# Purpose of the lesson:

To inform students about immunity, its types and forms. To acquaint them with non-specific protective factors of the organism (cellular and humoral). Explain phagocytosis and its stages, opsono-phagocytic index response and its role in diagnosis.

# Immunity

- greek, «*immunitas*» - exemption from obligations, privilege
- immunity – processes and mechanisms supporting inner stability of organism by protecting it from pathogens and other genetically foreign substances

# Types of immunity

- **Innate or species immunity** – organism is insensitive to antigen and passes this feature to next generation
- **Acquired immunity** - formed after exposure of the organism to microorganisms or other antigens, is not transmitted from generation to generation.

# Acquired immunity

Active and passive acquired immunity.

- Active immunity
  - natural
  - artificial
- Passive immunity
  - natural
  - artificial

# Immunity manifestation

- **Antibacterial**
- **Antiviral**
- **Antitoxic**
- **Antifungal**
- **Antiprotozoan**
- **Transplantation**
- **Antitumor**
- **Sterile and non-sterile**

**Non-specific and specific immunity**

# Sterile and nonsterile immunity

- **Sterile immunity** the complete elimination of pathogens from the body.
- Non-sterile immunity can not eliminate microorganism from the organism, in other words it exists only in presense of pathogen and disappears when the pathogen leaves macroorganism. Thus, it is also called infection immunity.  
Non-sterile immunity is observed in tuberculosis, syphilis etc.

## Specific immunity

- The activity of specific factors depends on the type of antigens that enter the body.
- A specific defense factor formed against any antigen cannot protect the body from other antigens, in other words, these factors have specificity.

## Types of non-specific immunity

- Non-specific defense factors can be divided into specialized and non-specialized, humoral and cellular.
- ***Specialize defense factors primary function is defense of organism, while the primary function of non-specialized factors (non-specific resistance) is not defense.***
- ***Humoral factors***- dissolved substances,
- ***Cellular factors*** consist of different cells.

## **Non-specialized defense factors or non-specific resistance**

- Skin and mucous membranes are outer barriers of organism.
- The integrity of skin and mucous membranes and their impermeability for foreign antigens is vital for defense:
- Alteration of integrity increases possibility of entering microorganisms.

# Non-specific humoral defensive factors

- There are many non-specific humoral defense factors in body tissues and blood.
- They usually have an antimicrobial effect or are involved in the activation of other immune factors.
- Non-specific humoral defense factors include secretory immunoglobulins, complement system proteins, lysozyme, C-reactive protein, transferrin, interferon and IFN.

# Lysozyme

- Lysozyme is an enzyme composed of 129 amino acids with molecular weight about 14 kD.
- It breaks down the glucoside bond between N-acetylmuramine acid and N-acetylglucosamine in the bacterial cell wall.
- As a result, the synthesis of the bacterial cell wall is disrupted and microorganisms turn into spheroplasts or protoplasts.

# Lysozyme

- Lysozyme is synthesized in monocytes, macrophages and neutrophils.
- It is found in relatively high concentrations in egg white, tears, saliva, sputum, nasal secretions, and blood serum.
- In humans, high levels of lysozyme are found in tissues - cartilage and stomach, in low concentrations - in the intestines, kidneys, liver, tonsils and brain.
- In healthy people, it is not detected in the cerebrospinal fluid. The concentration of lysozyme in tears is 100-160 times higher than in the blood serum.

# Complement

- About 130 years ago, V.Isayev and R.Pfeifer discovered that fresh blood serum obtained from animals has bacteriolytic properties.
- This antimicrobial serum factor was later called alexin or complement (Latin, complementum).
- The complement system consists of more than 20 thermostable and thermolabile components (C1, C2, C3, etc.) and makes up to 10% of the globulin fraction in the blood.

# Complement

- Activate by sequential interactive convertation of proteases.
- Complement has wide spectrum of biological activity and lysis of cells is the most important among them.

# Complement

- The system consists of 3 groups of proteins.
- The first and second proteins activate C3-components which is opsonin participating in phagocytosis.
- C3-C3b fragment activates formation of C5-C9 complex which in turn causes alteration of target cell membrane and its lysis. This complex is called membrane attacking complex(MAC).
- C3a and C5a have chemoattractant activity.
- C3a and C5a are anafilatoxins, in other words they cause mast cell and basophiles degranulation and development of allergic reactions.

# Activation of complement system

There are 3 pathways of complement activation:

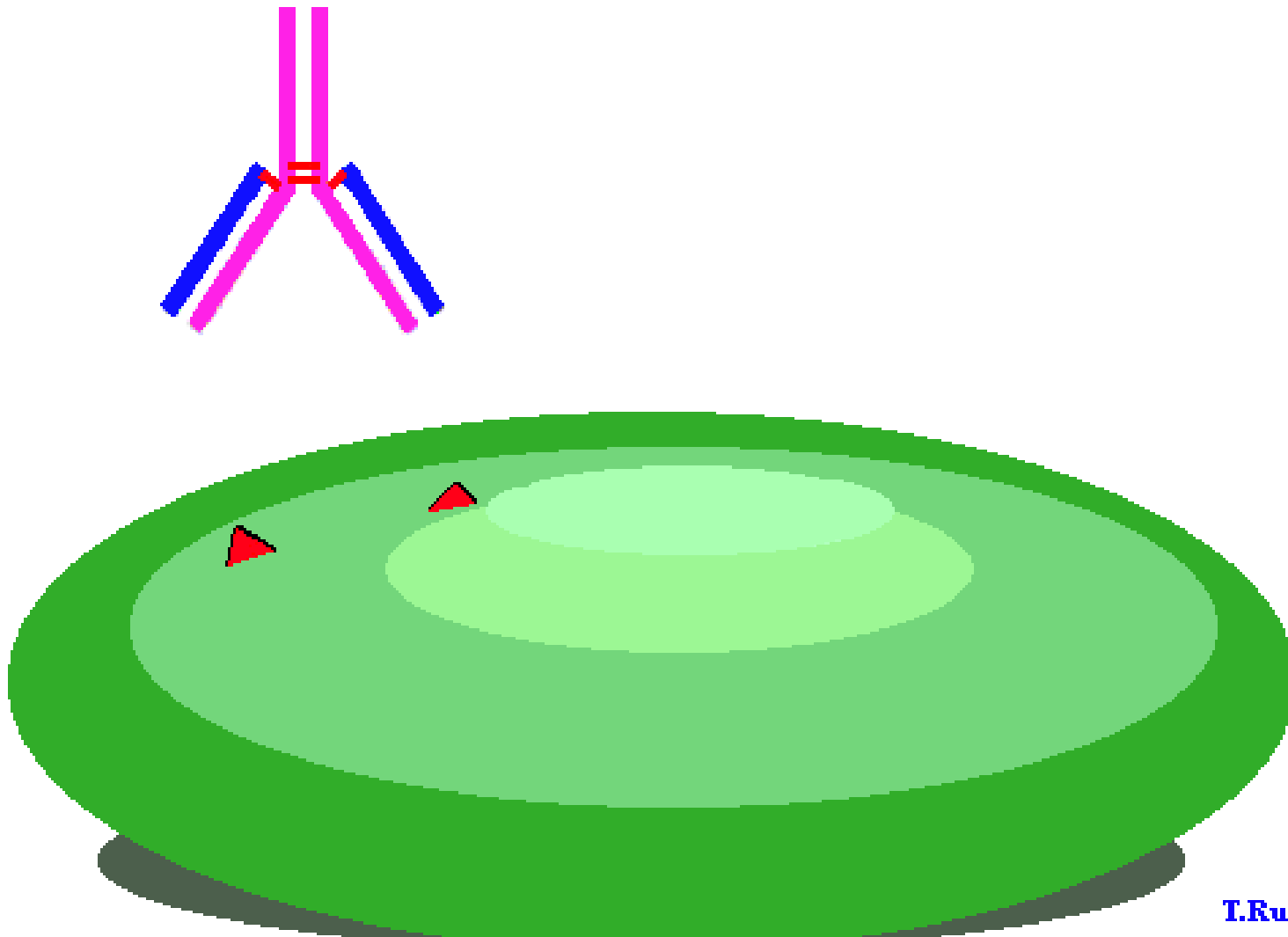
- **Classic**
- **Alternative**
- **Lectin**

# Activation of complement system

- ***Classic way*** begins connection of C1 component with antigen-antibody complex.
- After activation C1 component becomes enzymatically active and activates C2 and C4 components.
- C2a and C4b subcomponents released after breakdown of C2 and C4 components form protease complex which breaks down C3 component.
- Finally membrane attacking complex is formed.

# Activation of complement system

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# Activation of complement system

- The presence of antibodies is not required for alternative way of complement activation. This pathway is common in defense against gram negative microorganisms.
- Cascade reactions begin with the combination of an antigen (e.g., polysaccharide) with B, D, and P (properdine) proteins and the activation of component C3, followed by a formation of membrane attacking complex (MAC)

# Activation of complement system

- Activation of the complement by the lectin pathway also occurs without the participation of antibodies.
- It begins mannose binding protein binding with mannose of microbe cell wall. It causes activation of C4 component.  
The subsequent cascade of reactions is the same as in the classical way.
- Mannose-binding protein is a normal serum protein. It firmly attaches to the mannose on the surface of microbial cells and has the ability to opsonize them.

# C-reactive protein

- During acute inflammation the concentration of acute phase proteins in blood serum increases. This protein can react with C protein of Pneumococcal cell wall.  
Along with properdin, CRP can be an initiator of alternative activation of
- CRP levels increase in the blood of patients with various infectious diseases.
- Evaluation of its levels in rheumatism has high value in determining disease severity.

# Prostaglandins

- Prostaglandin synthesis is induced by microorganisms, hormone, complement components (C3b) etc.
- They induce migration and degranulation of neutrophils. At the same time they have pyrogenic activity

# Kinins

- Kinins are alkaline proteins. They are produced from kininogens of plasma and tissue as a result of plasma clotting and proteolysis.
- They reduce arterial tension, stimulate secretion of soluble factors by leucocytes.

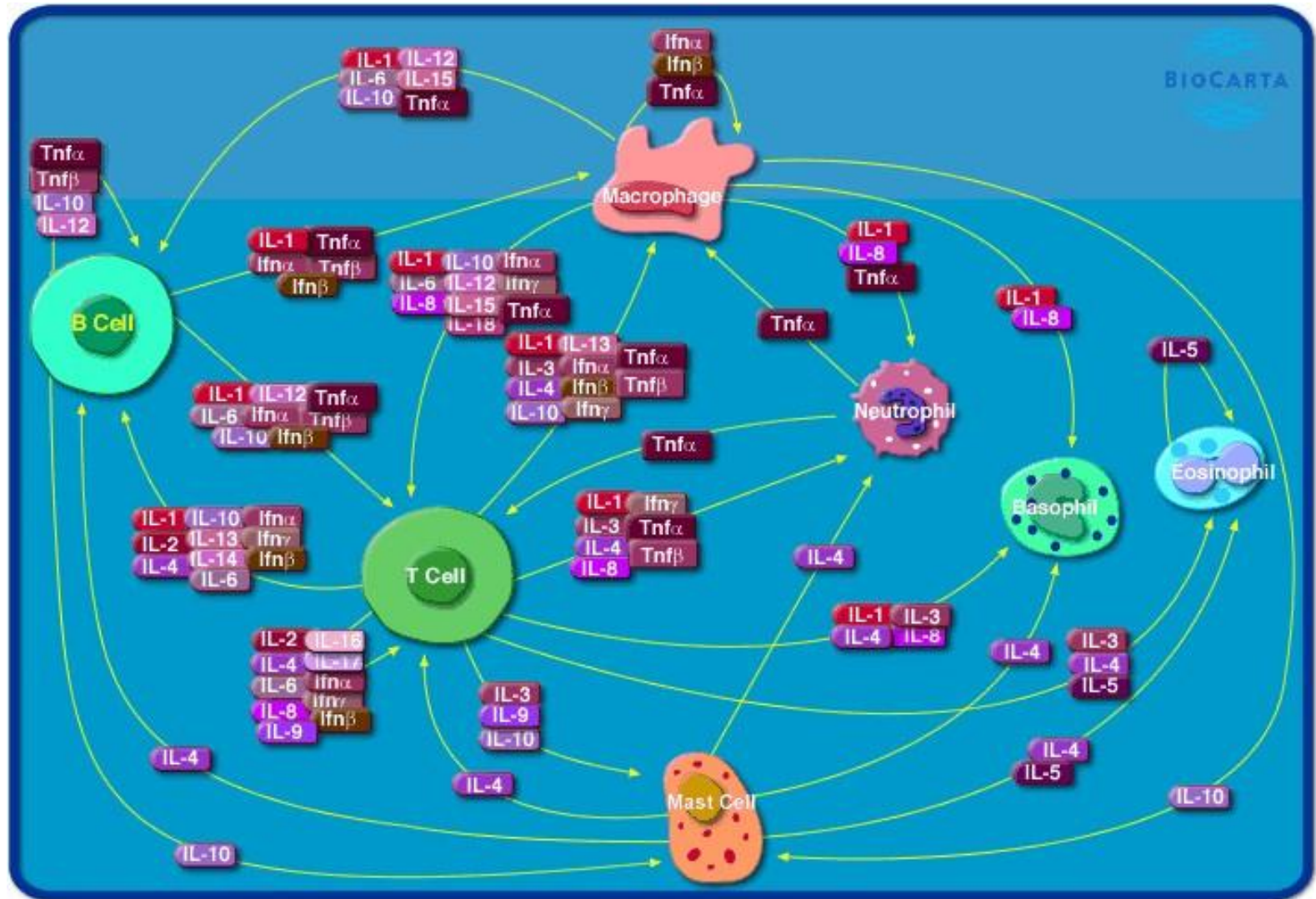
# Cytokines

- Cytokines are small molecular immune modulators synthesized by immune system cells and participating interaction between cells.
- They are not synthesized in absence of antigen stimuli.
- After antigen stimuli cytokine genes are induced and cytokines are produced.

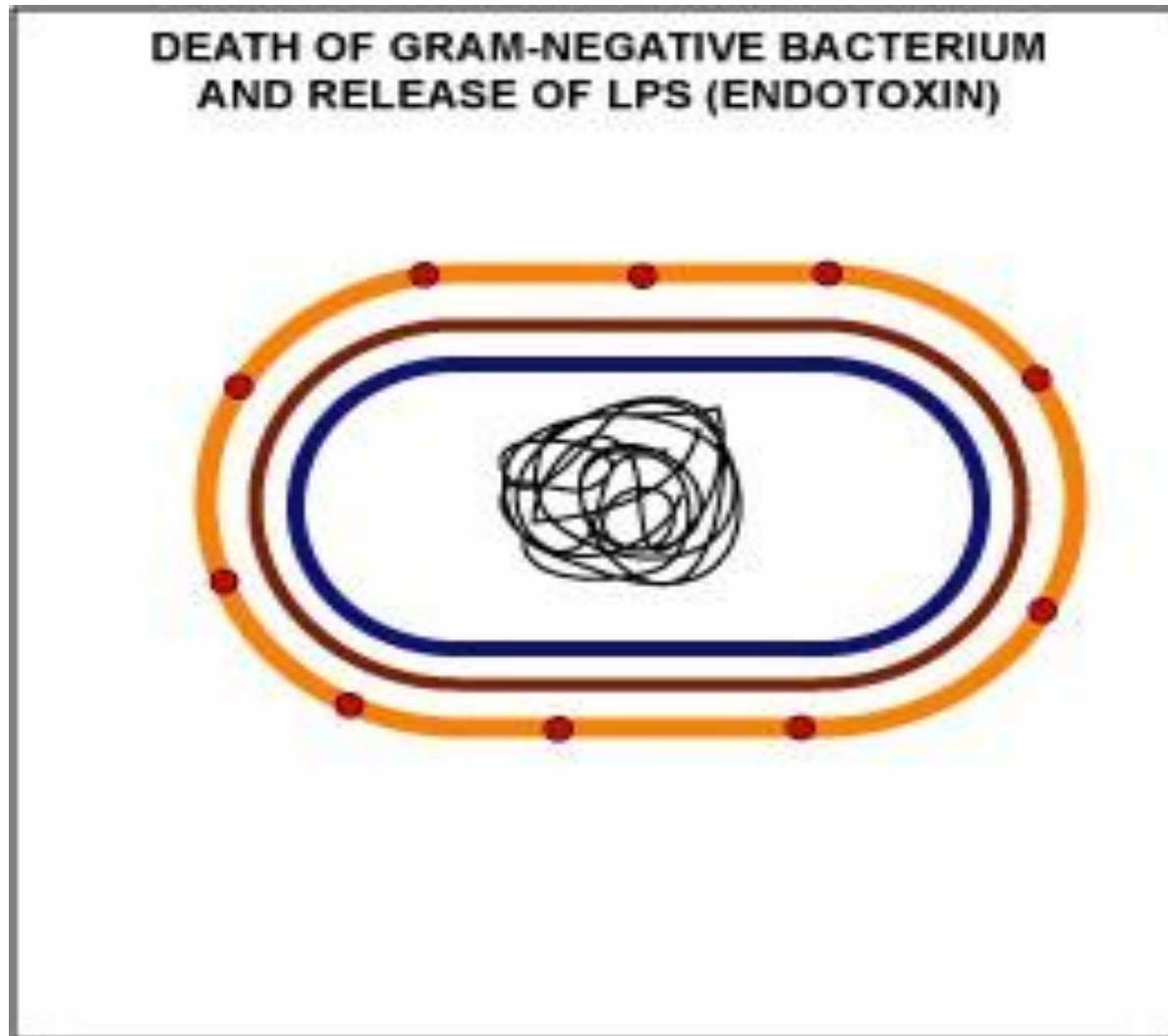
# Cytokines

- Cells express certain receptors which can interact with different cytokines;
- Cytokines do not accumulate in cells and released immediately after a certain stimulus;
- Cytokines act on producers and other cells;
- Cytokine regulation has cascade character – activation of cell by one cytokine stimulates production of another;
- Unlike the hormones of the endocrine glands, in most cases they are short-distance mediators – cytokine effects are manifested only in places of their release. However, a number of inflammatory cytokines (IL-1, -6, TNF  $\alpha$ , etc.) can have a systemic effect.

# Cytokines



# Induction of cytokine synthesis



# Cytokine classification

Depending on biological effects and structural features:

- interleukins (IL),
- interferons (IFN),
- Tumor necrosis factors( $\text{TNF}\alpha$ ),
- Colonystimulating factors,
- Chemokines

# Cytokine classification

Depending on their producers, cytokines have received different names:

- monokines synthesized by monocytes and macrophages,
- lymphokines synthesized by lymphocytes, etc.

# Lymphokines

- T-helpers are the main lymphokine producers.
- Antigen stimulated T helpers (Th) synthesize IL-2, differentiate to Th1 or Th2 lymphocytes.
- Th1 lymphocytes produce interferon, IL-2, TNF
- Th2 lymphocytes produce IL-4, 5, 6, 9, 10, 13.

## Classification based on function

- Immun pre-inflammatory mediators (IL-1, -6, -12,  $\alpha$ - TNF etc.);
- Immune inflammatory mediators (IL-5, -9, -10,  $\gamma$ - IFN etc.);
- Lymphocyte differentiatiaion and proliferation modulators (IL-2, -4, -13 etc.);
- Growth factors (IL-3, -7, QM-CSF etc.);
- Chemokines or cell chemoattractants (IL-8 etc.);

# Interleukins (IL-1)

Up to 20 interleukins is known.

- IL-1 is the first invented interleukin. Monocytes and macrophages are the main producers of IL1. Play a role nonspecific signal role in antigen presentation by macrophages to T lymphocytes.

# Interleukins (IL-2)

- IL-2 is one of the first studied mediators. Its main producers are T-helpers, and its main targets are activated lymphocytes (T and B) and natural killers.
- Stimulates the division of T-lymphocytes, the differentiation of T-killers, enhances the cytotoxic activity of natural killers.
- This cytokine is considered to be one of the growth factors of activated B-lymphocytes. It accelerates the synthesis of immunoglobulins.

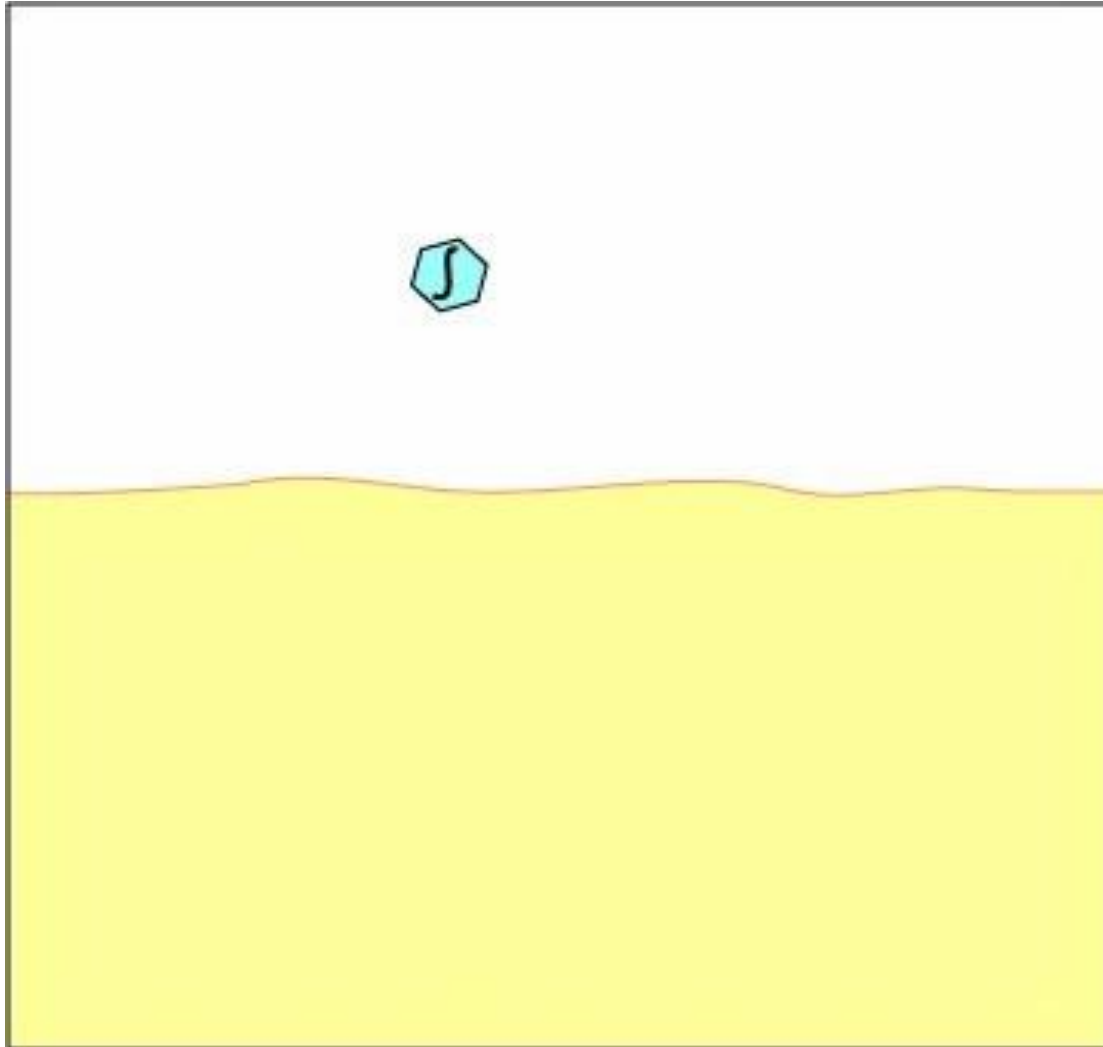
# Tumor necrosis factors

- *Tumor necrosis factors (TNFs) are so named because of their ability to induce the lysis of tumor cells. TNF- $\alpha$  and TNF  $\beta$  can bind to glycoproteins called  $\beta$ -lymphotoxins.*
- TNF  $\beta$  is also called  $\alpha$ -lymphotoxin.  $\alpha$ - and  $\beta$ -lymphotoxins are produced by T-killers.
- These cytokines bind to certain receptors on cell surface and activate apoptosis in target cells.

# Interferon

- Interferon (IFN) is synthesized by immunocompetent and somatic cells.
- It has species specificity, in other words, IFN of human origin is important only to humans.
- Viruses are the main interferon inducers. However, bacteria, fungi, mycoplasmas and other microorganisms, as well as their antigens and non-specific stimulants (phytohemagglutinin PHA) can induce interferon synthesis as well.
- Interferon suppress viral protein replication by affecting t-RNA

# interferon synthesis



# Interferons

Depending on cellular origin and inducing factors:

- Leucocytes (alfa),
- fibroblasts (beta) and
- immune (gamma) interferons:

# Alfa-IFN ( $\alpha$ -IFN)

- $\alpha$ -IFN are produced by leucocytes.
- $\alpha$ -IFN plays mediator role by acting on immune competent cells function.
- $\alpha$ -IFN activates macrophages, lymphocytes, nature killers.

# Beta-IFN ( $\beta$ -IFN)

Secreted by somatic cells (especially fibroblasts)  
after induction by viral infections.

# Gamma-IFN ( $\gamma$ -IFN)

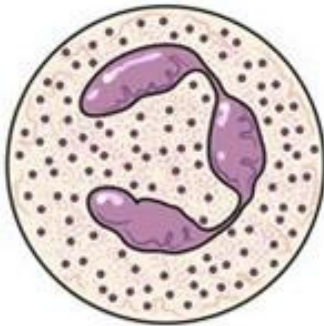
- Secreted by T- and B-lymphocytes after stimulation by mitogens and antigens.
- $\gamma$ -IFN decreases proliferation of leucocytes and antibody synthesis.

# Cellular factors of non-specific defense

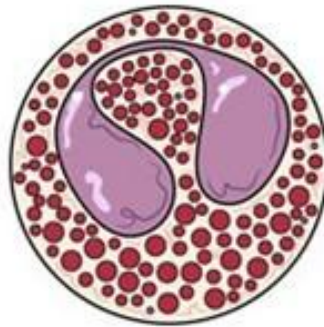
- Non-specific cellular defense is performed by phagocytes.
- 2 types phagocytes – micro- and macrophages exist.
- Neutrophils, monocytes and tissue macrophages form monocyte-phagocyte system.

# Phagocyte

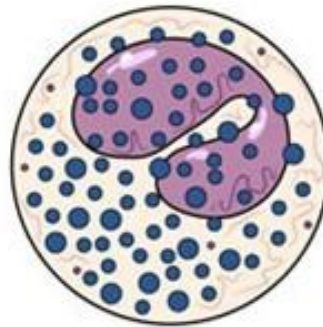
Neutrophil



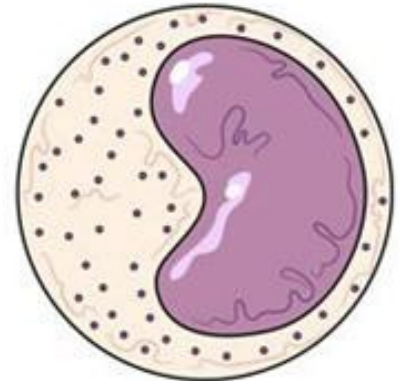
Eosinophil



Basophil



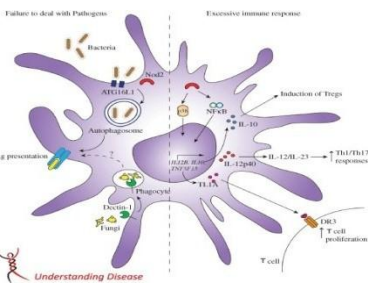
Monocyte



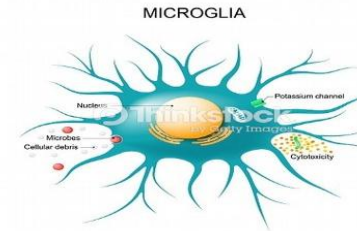
## Other cells with phagocytic activity

- endothelial cells of blood and lymph vessels,
- cells of the pleural and peritoneal membranes,
- reticuloendothelial cells of the liver (Kupffer cells),
- dendritic cells of the lymph nodes (Langerhans cells),
- histocytes,
- fibroblasts, etc.

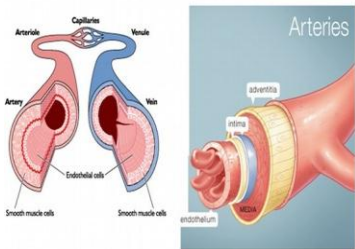
# Other cells with phagocytic activity



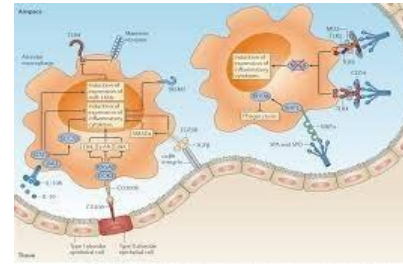
**Kupffer cells**



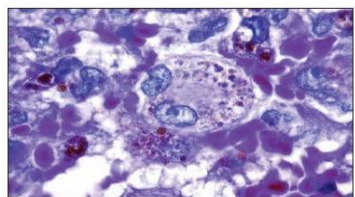
**Microglial cells**



**Endothelial cells**

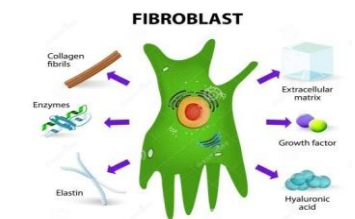


**Alveolar macrophages**

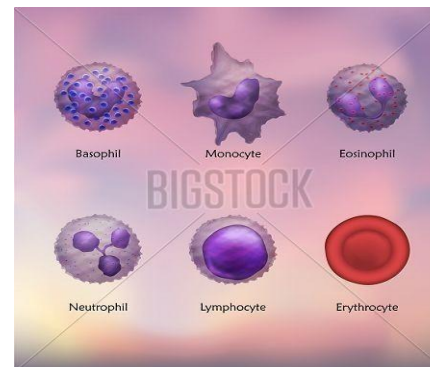


**Langerhans cells(skin)**

Figure 2. Hematoxylin eosin staining demonstrates phagocytosis of hemopoietic cells by Langerhans cells recognizable by their irregular nuclei with a round or elongated contour, frequently with cleavage and indentation, loose and well-distributed chromatin.



**Fibroblasts**

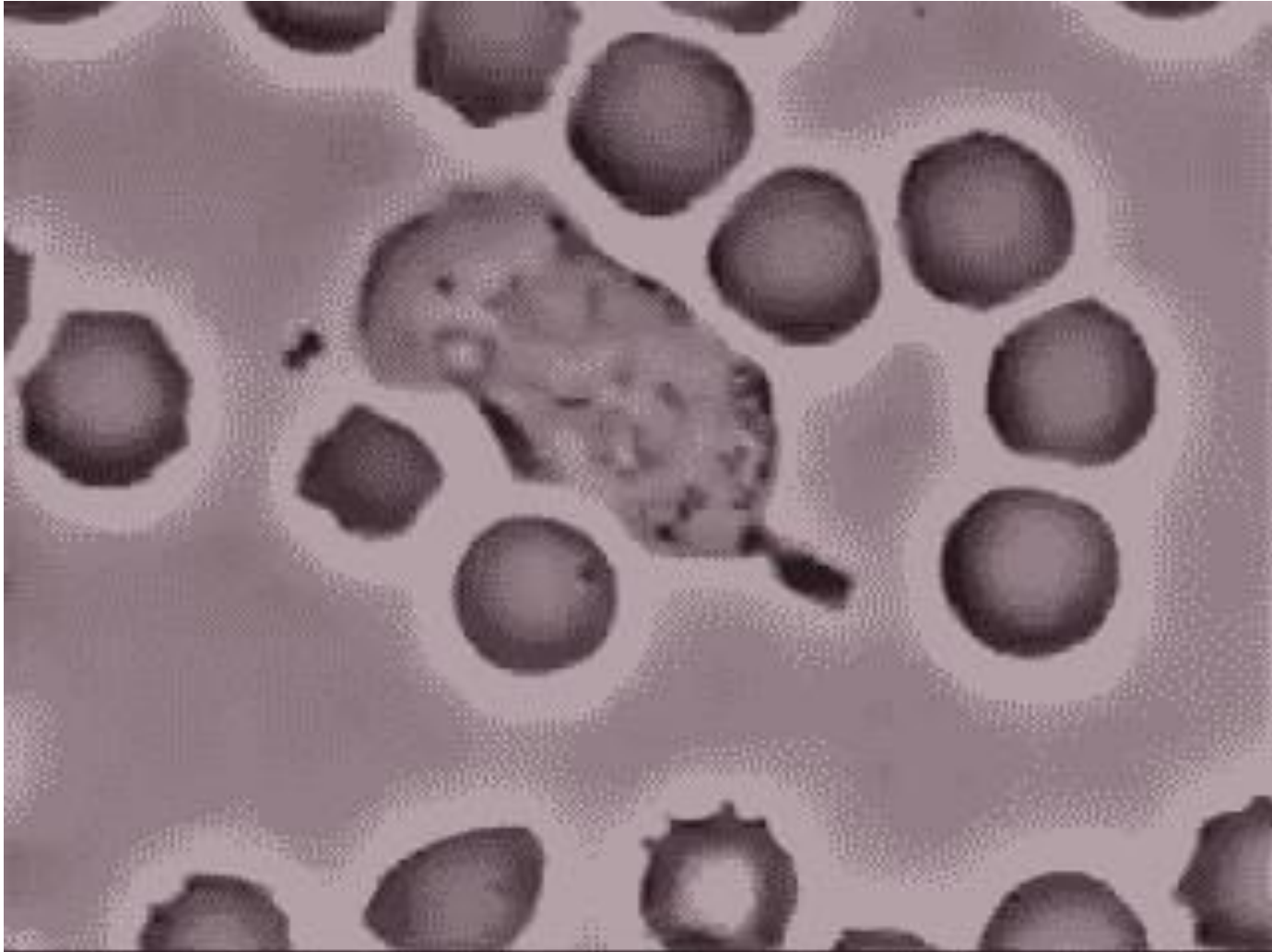


**Mesenchymal cells**  
**Osteoclasts (bone)**  
**Dendritic cells**

# Phagocytes

Phagocytosis (greek, *phagos*-engulf, *cytos*-cell) absorption and neutralization of microorganisms, cells with altered antigenic features, foreign bodies by neutrophils and macrophages.

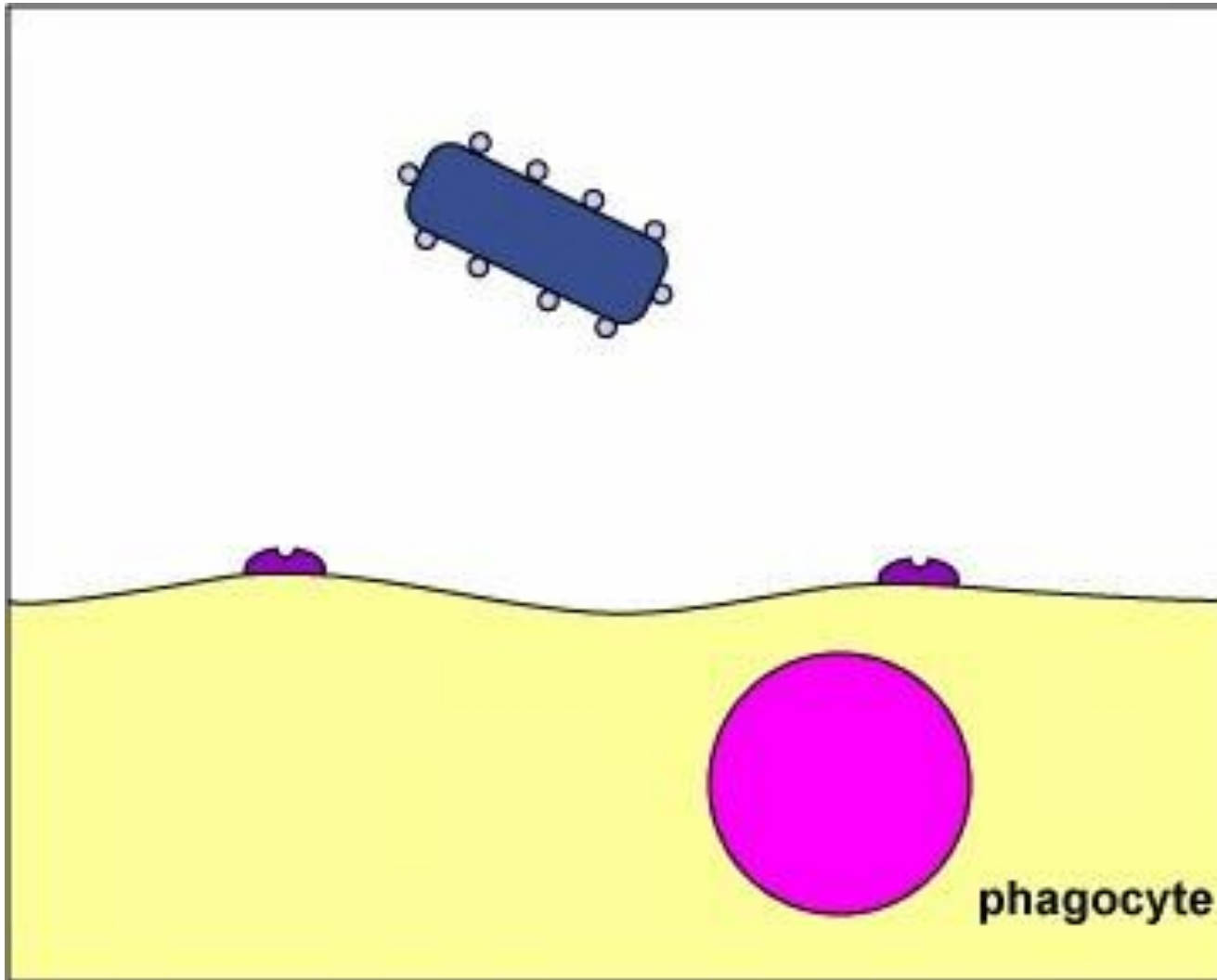
# Phagocytosis



# Steps of phagocytosis

- The process of phagocytosis has three steps- migration, ingestion and killing (killing).
- The process begins with the migration of phagocytes to the object of phagocytosis.
- It occurs through chemotaxis of phagocytes induced by chemoattractants - metabolic products of microorganisms, tissue and cellular debris etc.

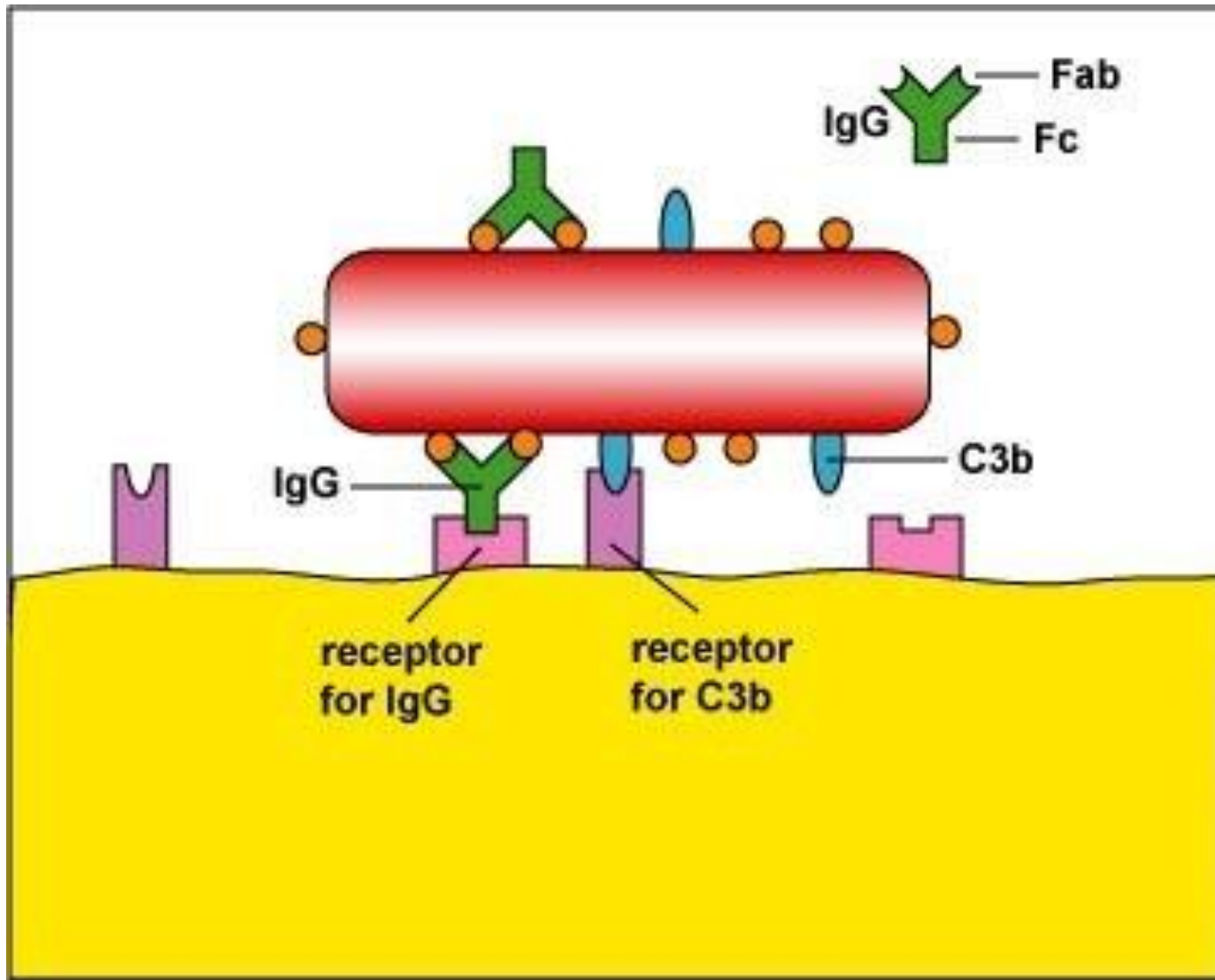
# Phagocytosis process



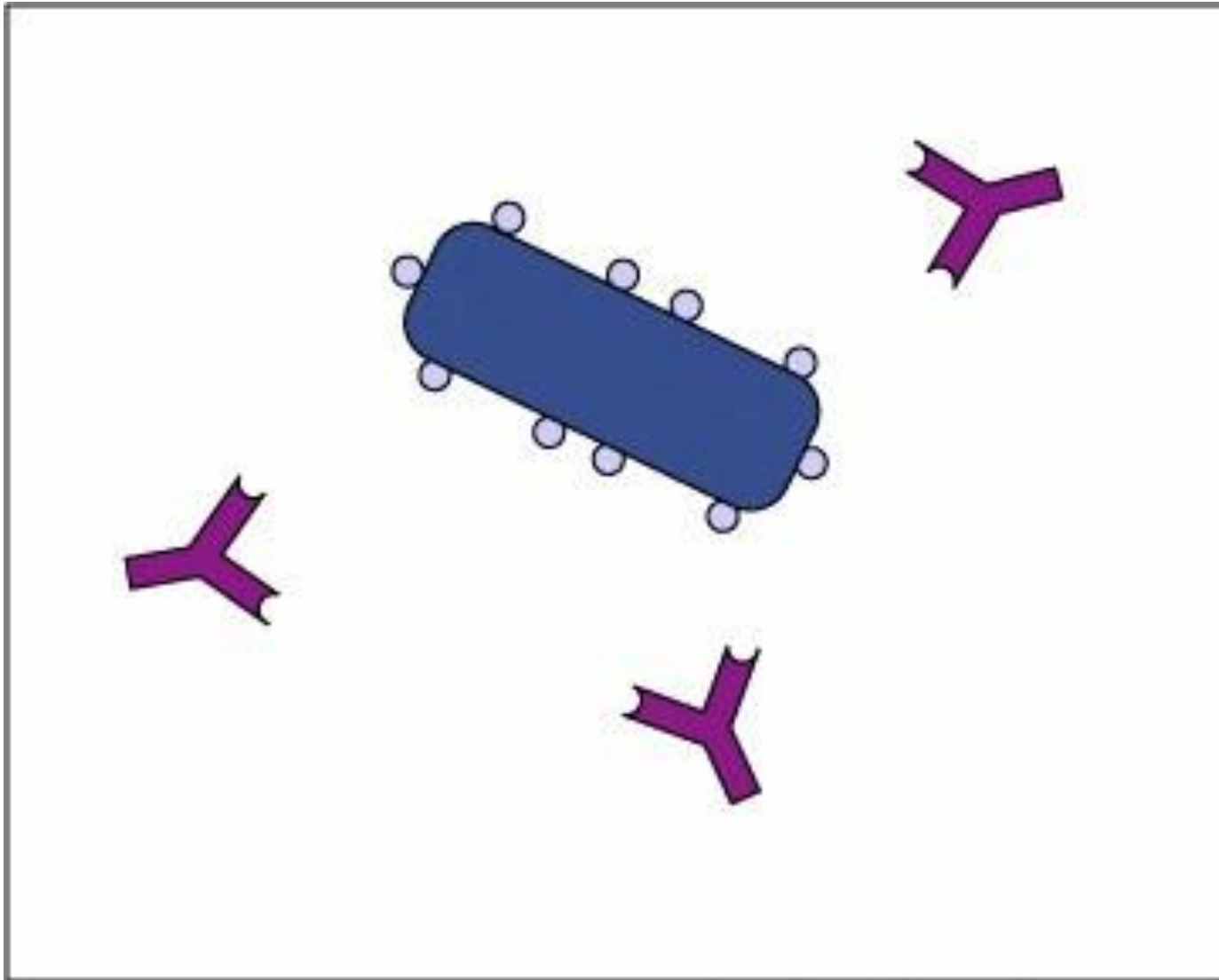
# Opsonization

- Opsonization – attachment of antibodies and complement to the object of phagocytosis – plays an important role in phagocytosis.
- Opsonized object is easily recognized by phagocytes as they have special receptors for opsonins.
- Phagocytosis may occur without opsonization as well however with low efficacy.

# Opsonization



# Opsonization



# Mechanism of phagocytosis

- Objects attached to the phagocytes membrane are surrounded by **pseudopods** resulting with formation of ***phagosome (vacuoles)*** in protoplasm.
- Then, after fusion of phagosome with lysosome ***phagolysosome*** is formed and the object is processed and disintegrated by phagocyte enzymes.
- Complete digestion of engulfed microorganism by phagocytes is called ***complete phagocytosis***.

# Mechanism of phagocytosis

- The processing of some microbes in phagocytes occurs without opsonization.
- At some conditions even activated phagocytes can not process these objects resulting in ***incomplete phagocytosis*** characteristic for granulomatous infections (tuberculosis, brucellosis etc.)

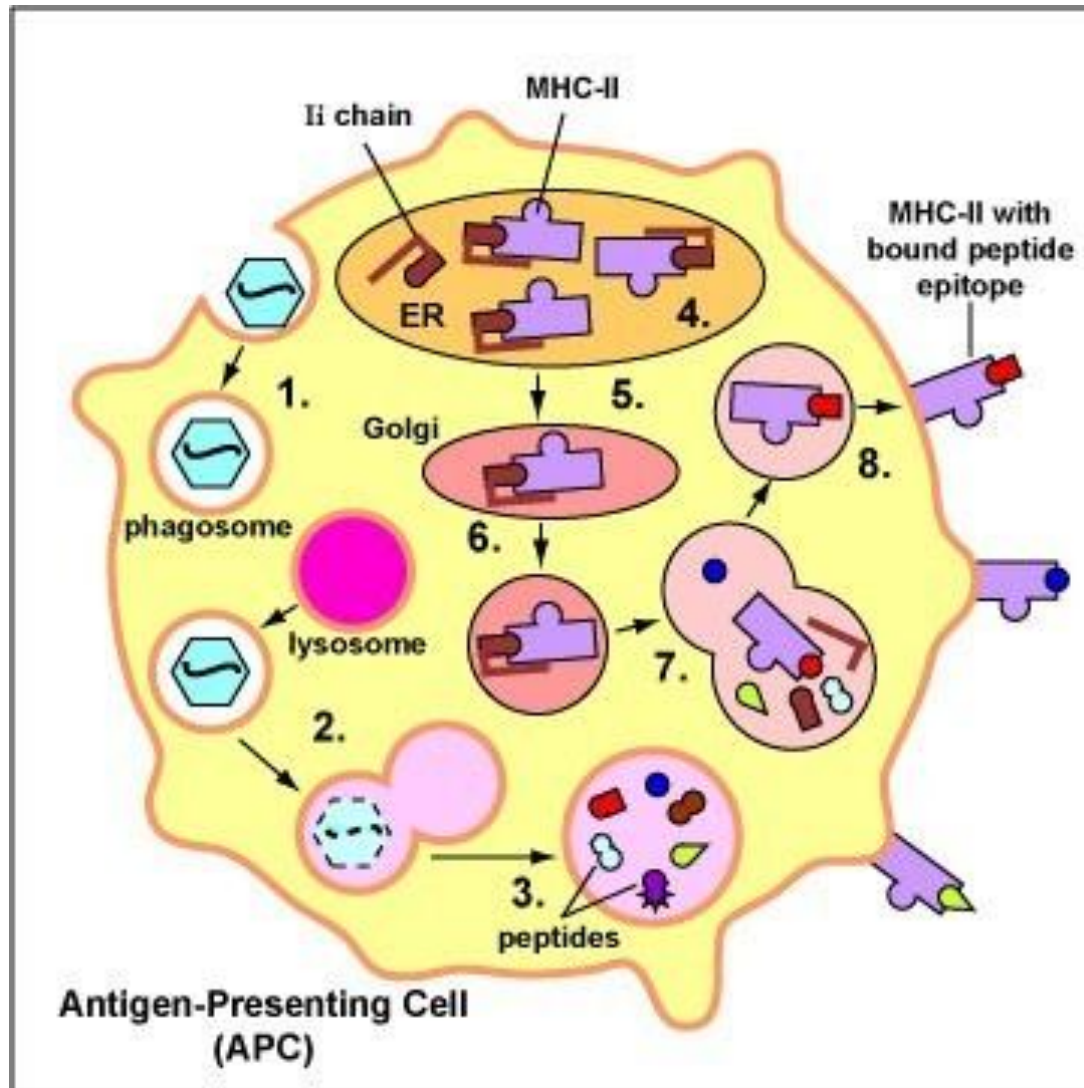
# Killing of microorganisms in phagocytes

- Various mechanisms are involved in killing of microorganisms in phagocytes: oxygen-dependent and non-oxygen-dependent mechanisms.
- The oxygen-dependent mechanism begins immediately after phagosome formation and destroys objects inside the phagocyte with oxygen radicals.
- Absorption of the object is accompanied by a "respiratory explosion" in phagocytes, resulting in the formation of free oxygen radicals - superoxide radicals and hydrogen peroxide.

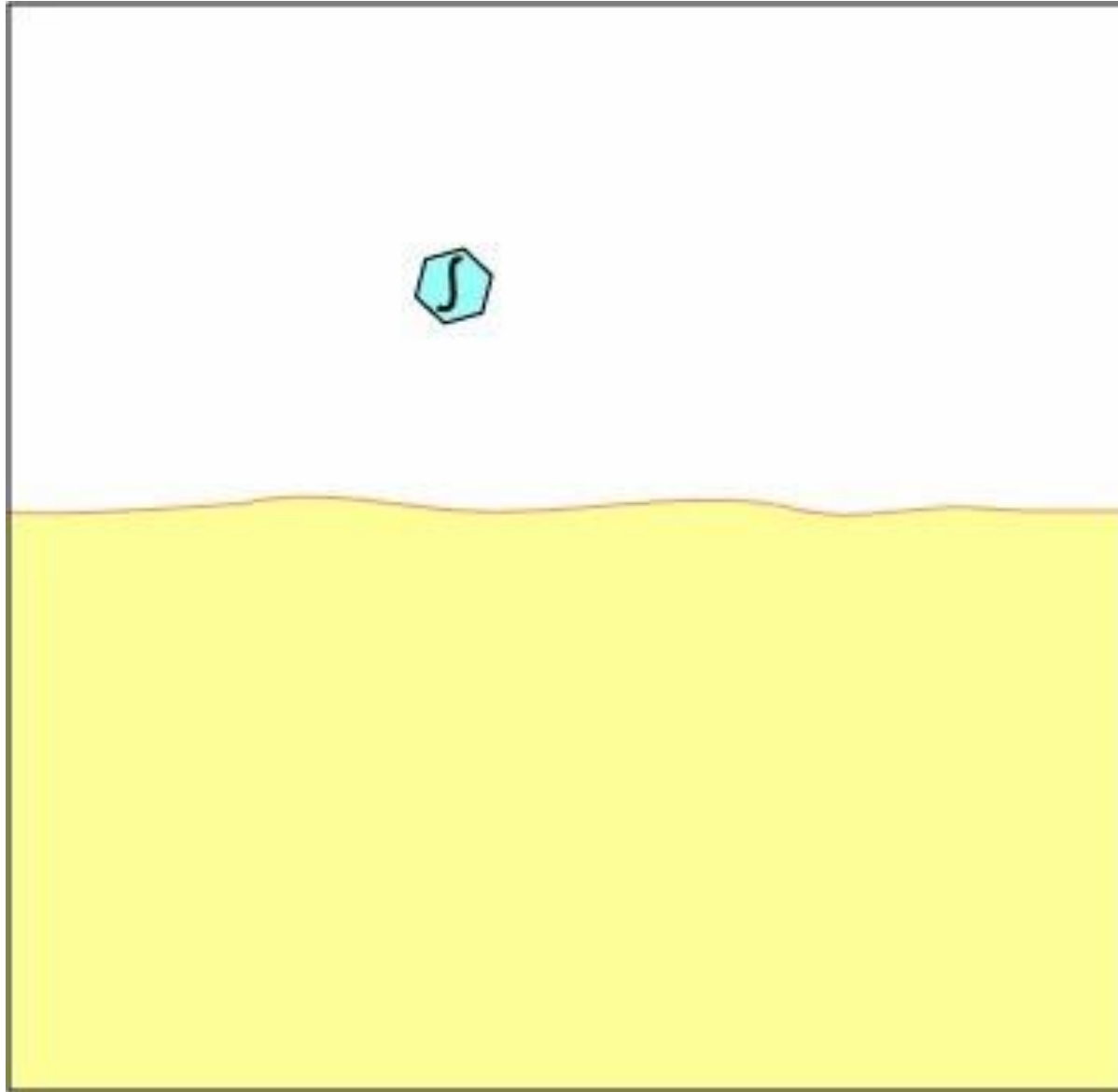
# Killing of microorganisms in phagocytes

- Oxygen dependent (free oxygen radicals -  $O_2^-$ ,  $1O_2$ ,  $OH$ ,  $OCl^-$ ,  $HO^-$ ,  $H_2O_2$  etc.)
- Oxygen non-dependent - lysosome enzymes (lactoferrin, lysozyme, cation proteins, defensin, elastase, collagenase etc.) act on object after phagolysosome formation.

# The processing of microbes in phagocytes



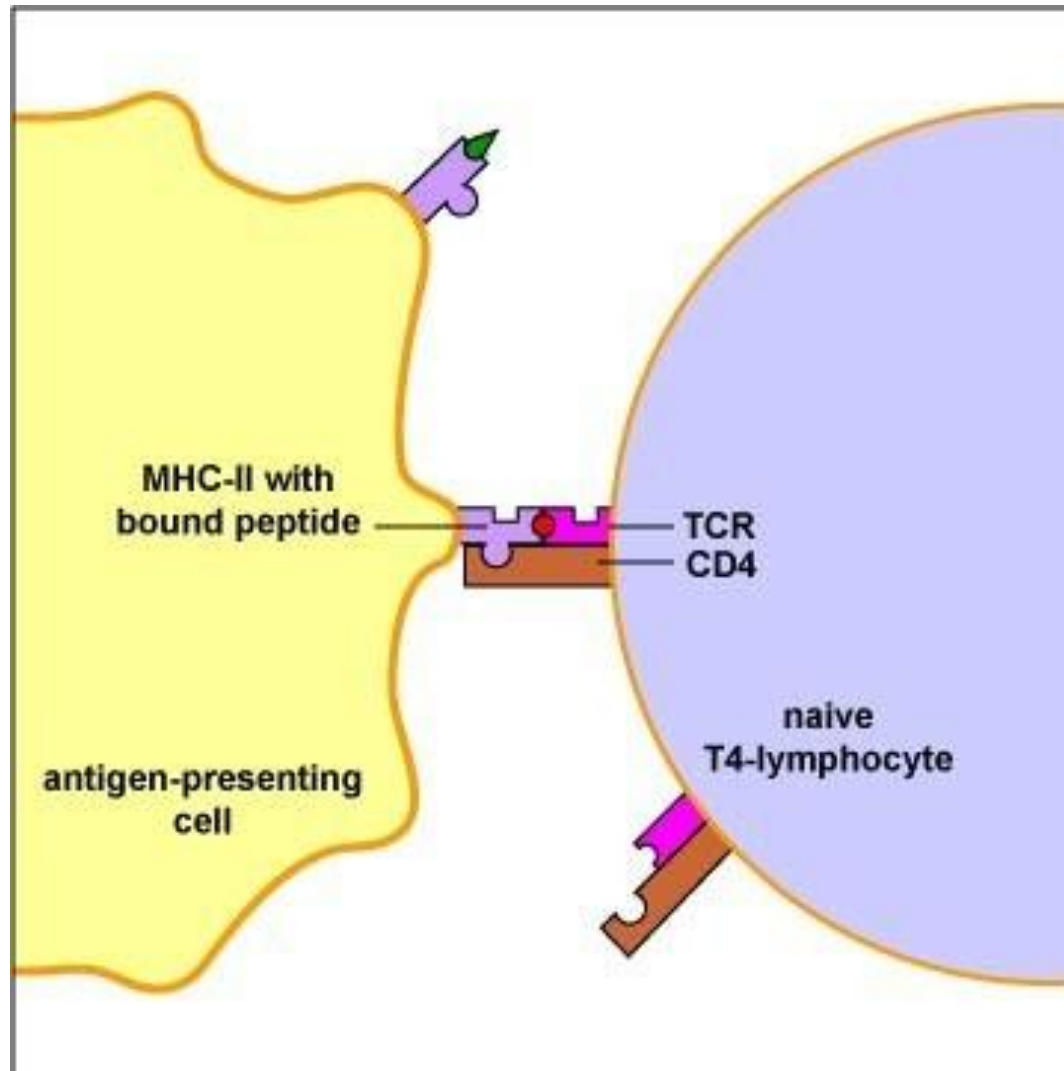
# Processing



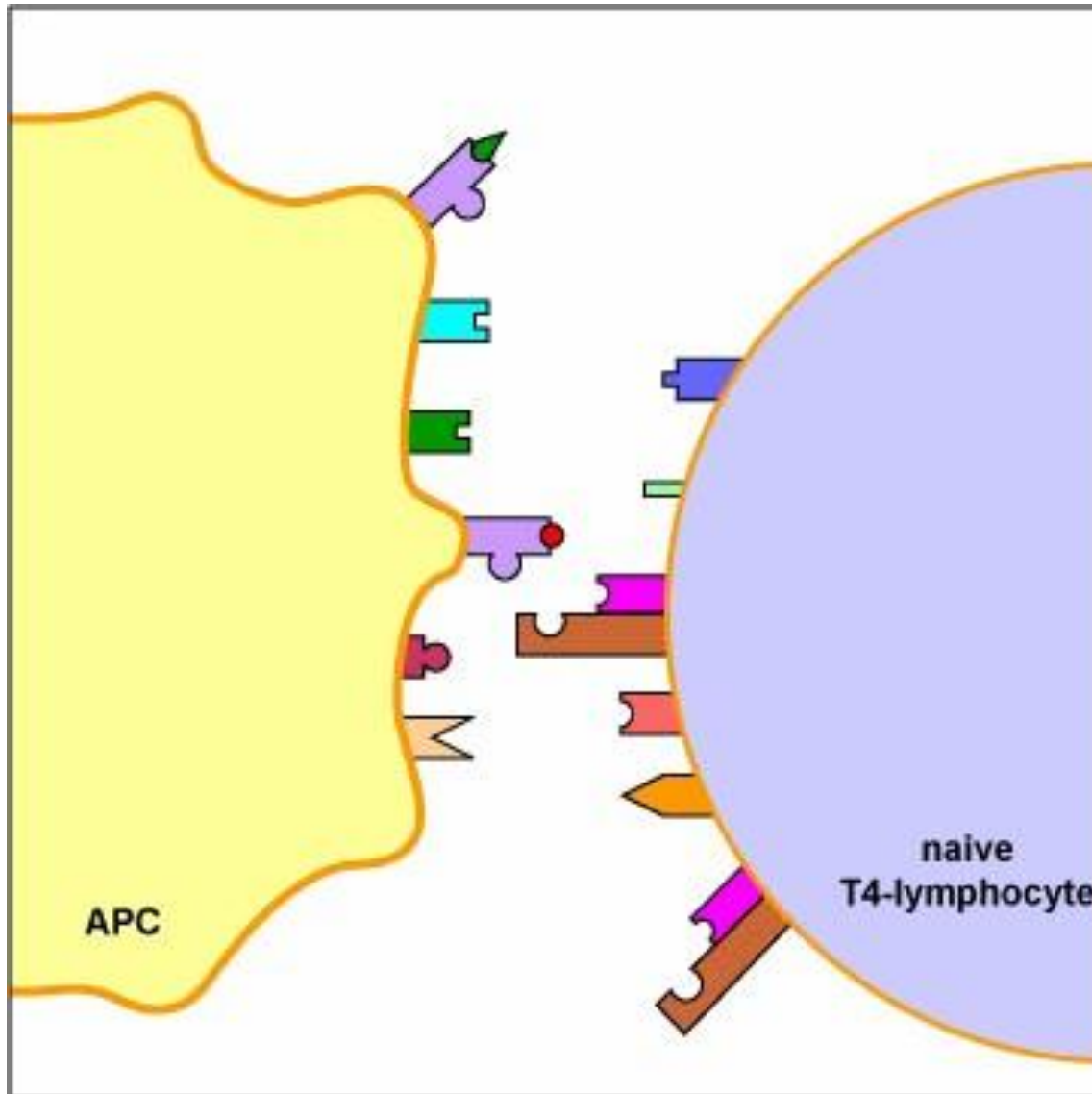
# Antigen presenting cells (APC)

- Functionally monocytes and macrophages have 2 subpopulations:
- first- perform only phagocytosis, second – phagocytosis and presentation of antigen to lymphoid cells.
- The latter cells called antigen presenting cells (APC) process antigen, present it to T and B-lymphocytes thus participating information of specific immunity.

# Presentation

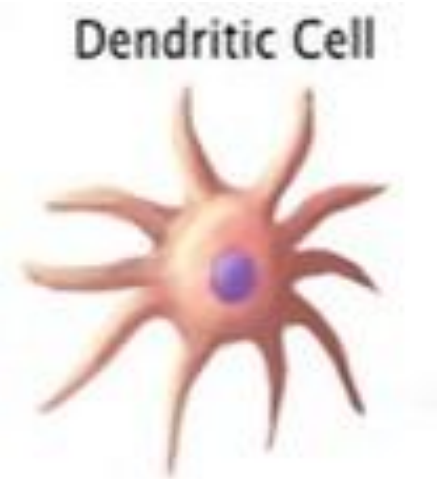


# Presentation



# Dendritic cells

- Dendritic cells– “tree like” (name “dendritic”) li are located in lymphoid and barrier tissues – especially in skin (Langerhans cells), lymphatic nodules (interdigital cells), thymus.
- MHC II complex proteins are expressed on their surfaces. ekspressiya olunur. Being the most active APC they can engulf antigen by endocytosis, process it and present to T-helpers in complex with MHC II.



# Eosinophils

- Eosinophils – granular leucocytes located in blood, connective tissue functioning as antibody dependent cellular cytotoxicity (ADCC) effectors.
- They accumulate at sites of helmynt invasion and mediate ADCC.
- They recognize parasites through receptors against IgA and IgE antibodies bound to helmynts.
- Activated they release toxic substances causing death of helmynts.



# Basophils

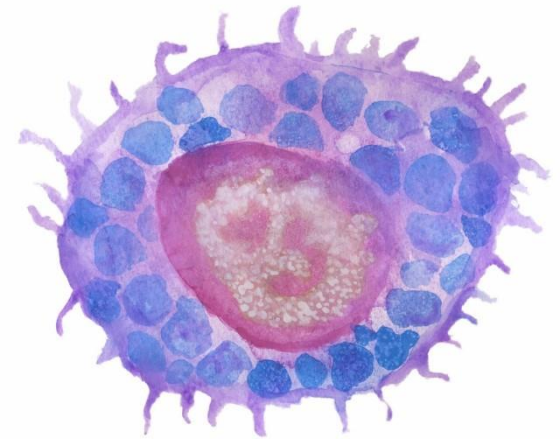
- Basophils are another granular cell type of nonspecific immunity circulating in blood.
- Two populations of basophils are distinguished – located in mucous membranes and connective tissues.
- High number of basophils in skin participate in skin associated immune responses.

Basophil



# Mast cells

- Mastocytes are myeloid cells located in barrier tissues mucous membranes and subcutaneous tissues.
- Depending on biologically active synthesized by mast cells they are divided to 2 subpopulations – mucous membrane and connective tissue mastocytes.



# Erythrocytes and platelets

- Erythrocytes participate in the immune defense by producing erythropoietin stimulating hematopoiesis maturation of other immunocompetent cells.
- Platelets, which produce the majority of serotonin, can also be classified as defense cells, as they participate in the fight against cancer.



# Determination of functional activity of phagocytic cells

- Functional activity of phagocytes is evaluated based on their ability to phagocytosis, degranulation, killing, generation of active oxygen forms.
- For this purpose, phagocytic activity, phagocytic number, opsono-phagocytic index, nitroterazole test (NTA-test) are performed.

# Phagocytosis activity and phagocytic index

- ***Phagocytosis activity***– the relative number of cells involved in phagocytosis.
- The patient's leukocytes are incubated with various microorganisms or other particles (latex, etc.).
- Smears prepared from this mixture and stained by the Giemsa method, 100 leukocytes are examined under microscope taking into account the number of phagocytes with engulfed microbes.
- **Phagocytic index** -the average number of microorganisms absorbed by one phagocyte is evaluated at the same smear.

## Evaluation of phagocytic activity of leucocytes

- 0.1 ml of examined blood is poured in a test tube containing 0.2 ml of 2% sodium citrate and mixed.
- 0.05 ml of microbial suspension(0.5 ml of microbial cells / ml) is added
- The mixture is incubated at 37 ° C for 30 minutes.
- The mixture is centrifuged at 2000-3000 r/min, the sediment is removed from the leukocyte layer by means of a pasteurized pipette.
- Smears (3-5 pieces) are made, coloured by Giemsa and examined under the microscope. 100 leukocytes are examined taking in account the number of engulfed microbes. Obtained results are given in percentages.

# *in vivo* phagocytosis experiment

- 2-3 ml of sterile meat-peptone broth is injected into the abdomen of a white mouse.
- After 3-4 hours, 0.5-1 ml of staphylococcal suspension (2 billion/ml) is injected in the same area.
- After 10-15 minutes, the fluid is removed from the abdomen, smear is prepared and stained with methylene and examined under the microscope.
- Leukocytes are stained blue while staphylococci appear dark blue.
- The percentage of leucocytes engulfed Staphilococci is counted by examination of 100 100 leukocytes.



# ***Determination of opsonization index***

- The activity of phagocytosis varies depending on the amount of opsonins in the blood. In order to evaluate opsonin number opsonization index is used.
- Phagocytosis tests are performed both in patient and control plasma. Phagocytosis activity is evaluated in both test tubes.
- The ratio of phagocytosis activity of patient and control plasma is called opsonization index.
- Opsonization index is high in presense of opsosnins in patient plasma. Thus, high opsonization index indicates positive prognosis of disease.

# Evaluation of killing activity of phagocytes

- During evaluation of ***killing activity*** the numbers of phagocytes and microorganisms are known in advance.
- Based on the changes in the number of microorganisms before and after phagocytosis, it is possible to make conclusion about the ability of phagocytes to kill (kill microorganisms).
- The number of microorganisms in the phagocytic mixture is determined by cultivation in appropriate nutrient media.

# Detection of oxygen active forms

- Ability to produce  $H_2O_2$  is evaluated. Production of  $H_2O_2$  depends on activity of myeloperoxidase system. ***Nitrotrazole a (NTA)-test*** is the most common test used for this purpose.
- The principle of the test is based on reduction nitrotrazole to formazane in presense of  $H_2O_2$ .
- Patient blood is incubated with nitrotrazole at  $37^{\circ}C$  for 20 . Formazane inclusions formed in phagocytes are counted and percentage of formazane positive cells is calculated.

# Nitrotriazole test

- The patient's blood is incubated at 37 ° C for 20 minutes in the presence of nitrotriazole.
- Formazane inclusions (granules) formed in phagocytes are determined by microscopic examination.
- The percentage of formazan positive cells is calculated.
- The normal range is 10-30%.

# Parameters characterizing phagocytosis:

- **phagocytosis number:** normal range 5-10 microbe cells. Depicts engulfing ability of neutrophils.
- **Phagocytic index:** normal range 65-95%. Phagocytic index is the percentage of neutrophils participating in phagocytosis.
- **Number of active phagocytes:**  $1,6-5,0 \times 10^9$ . – the absolute number of leucocytes in 1L blood performed phagocytosis.
- **Index of phagocytosis completion** – killing ability of phagocytes. Normally higher than 1.
  - Neutrophils activity is high at the beginning of inflammation.
  - Decrease of neutrophil activity indicates chronic and autoimmune processes.