LECTURE III

The knowledge about infection. Pathogenesis and diagnostic methods of infection diseases caused by different microorganisms (bacteria, fungi, protozoa, viruses). Immunity, its types. Innate (non-specific) and acquired (specific) immunity

Infection or infectious process

- ***Infection or infectious process*** cover pathological process occurring in macroorganism as a result of entry and reproduction of microorganism.
- The similar processes caused by protozoans, helminthes and insects are called invasion (lat, *invazio* – attack).
- The interaction of microorganisms with macroorganisms in the infectious process manifests itself pathogenetically and clinically as an infectious disease.

Infectious process conditions

- **Pathogenic microorganism**
- **Sensitive macroorganism**
- **Environmental conditions**

The role of microorganism in infectious process

- ***Saprophytic microorganisms*** **live in environment, human and animal organisms as commensals without causing disease** (greek, *sapros* – decay and *phyton* - plant).
- ***Pathogenic microorganisms*** (lat, *pathos* – suffering, *genos* - origin) enter sensitive macroorganism and cause infectious disease.
- Opportunistic microorganism can cause disease only under certain conditions. Their ability to cause disease is dependant on host macroorganism status.

Pathogenicity and virulence

- **Pathogenicity** is ability of microorganism to cause pathological prcess or disease. Pathogenicity is genetic feature of microorganims and specific for the majority of microorganismsç in other words, Patogenlik hər bir mikroorqanizm növünün genetik əlamətidir və əksər patogenlər üçün spesifik xarakter daşyır, each pathogenic microorganism causes specific disease.
- Pathogenicity may vary within the same species. The degree of pathogenicity is expressed in virulence (Latin, virulentus - toxic).
- For viruses, the term "infectivity" is used instead of "virulence".

Change of virulence

- Due to virulence a certain microorganism strains can be classified as strains with high, weak virulence and avirulent.
- Change of virulence – weakening or strengthening may be phenotypic or genotypic. Once the factor causing the change of virulence disappears, the virulence returns to its previous level.
- If the virulence change is due to genetic factor it is passed from one generation to another.

The factors influencing virulence

- Cultivation of microorganisms under unfavourable conditions, long-term cultivation on artificial media, passage in animal organism with weak sensitivity, impact of physical and chemical factors may cause weakening of virulence.
- Stabile weakening of virulence – attenuation is used in vaccine preparation.
- Passage of microorganism in organism of sensitive animal may strengthen the virulence. It may be due to selection of virulent population of microorganisms.

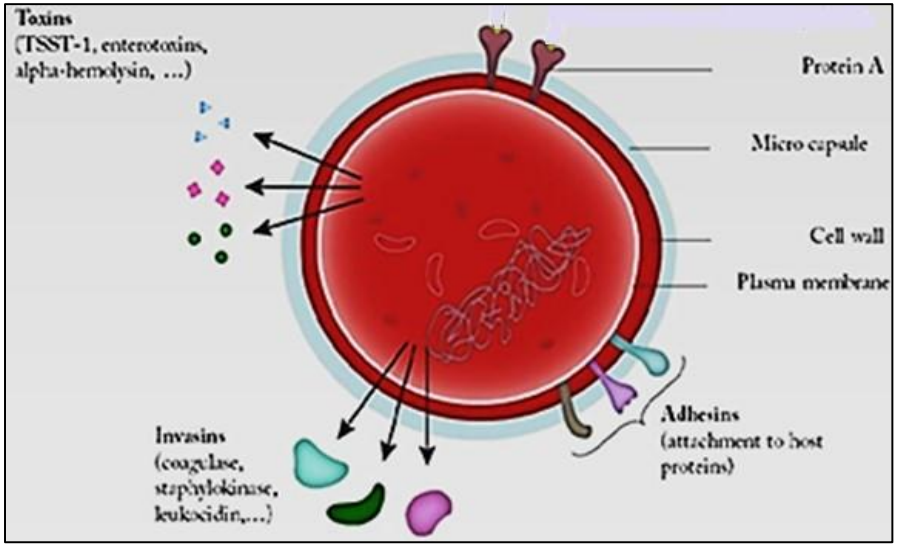
Virulence of microorganisms in the laboratory is usually assessed in laboratory animals, especially white mice. For this purpose, lethal and infectious doses are determined.

Lethal dose – the lowest number of microorganism or toxin causing death of certain number of animals over a period of time.

- ***Absolute lethal dose***(DCL - *dosis certa letalis*) – the lowest number of microorganism or toxin causing death of 100% animals.
- ***Minimal lethal dose*** (DLM - *dosis letalis minima*) – the lowest number of microorganism or toxin causing death of the majority (approximately 90%).
- ***Median lethal dose*** (LD_{50}) – the number of microorganism or dose of toxin causing death of a half of experimental animals. This dose is commonly used for evaluation of virulence.
- **Infective doses are** ID_{100} and ID_{50}.

Pathogenicity factors of microorganisms

- Pathogenicity of microorganisms is determined by **pathogenicity factors**. The presence of these factors distinguishes pathogen microorganisms from saptophytes.
- Pathogenic factors include the **morphological structures, enzymes and toxins** of microorganism cells.
- These factors enable entry, adhesion on tissue and cells of organism and protection of microorganism from defense system of macroorganism.

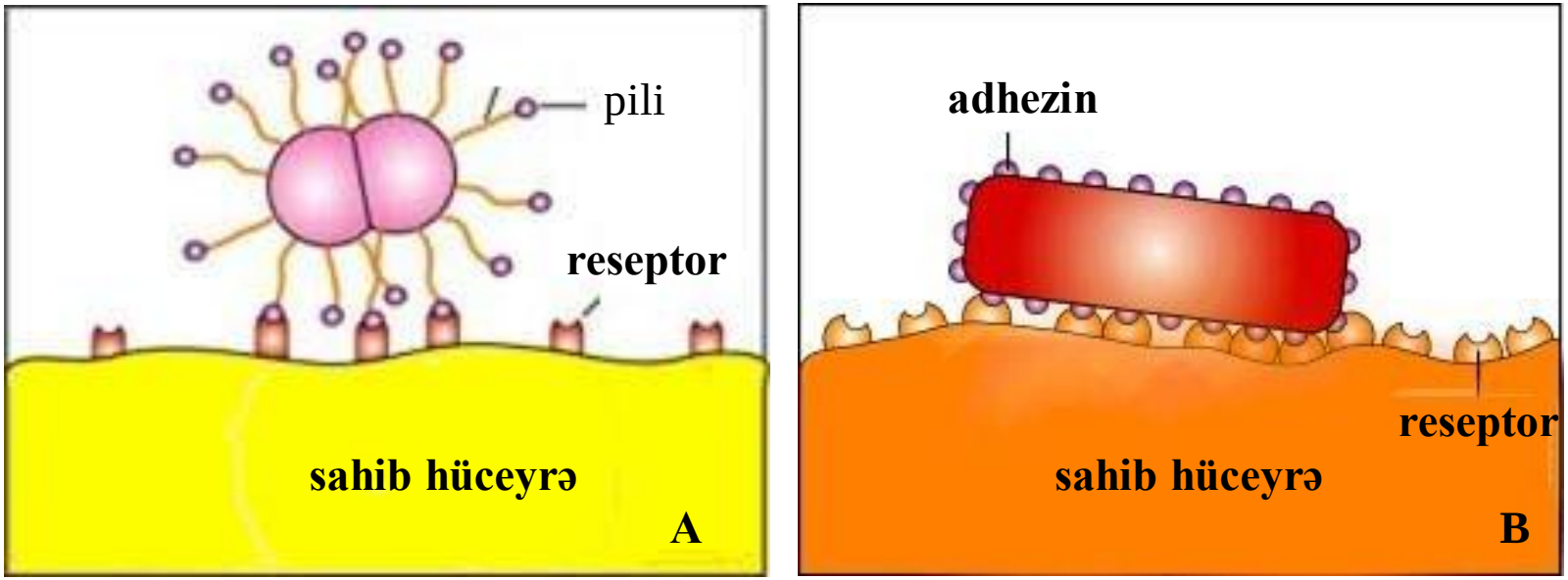


Pathogenicity factors of microorganisms

- ***Adhesion***– *specific connection of microorganism to sensitive cell.*
- **Colonization-** multiplication of microbe on curface of sensitive cell.
- **Penetration** – ability of some pathogens to enter in cells(epithelial, leucocites, lymphocites etc.).
- **Invasion**– entry of microbe through mucous membrane and connective tissue into necessary tissues (neuraminidase, hyaluronidase)

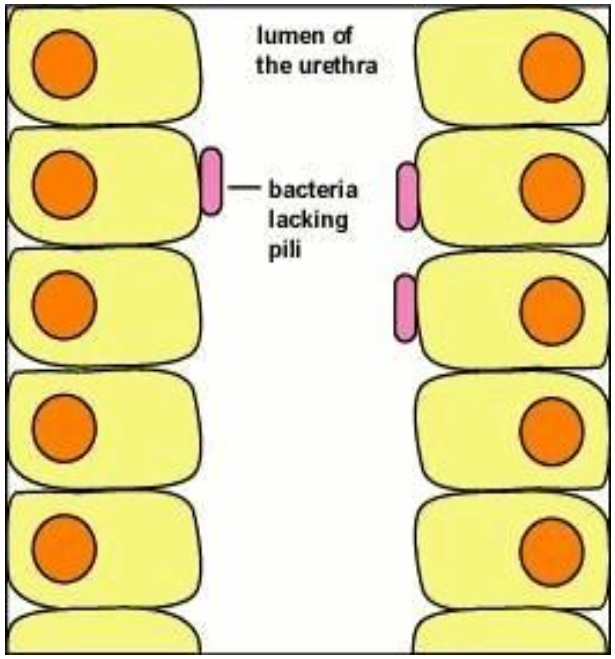
Adhesion

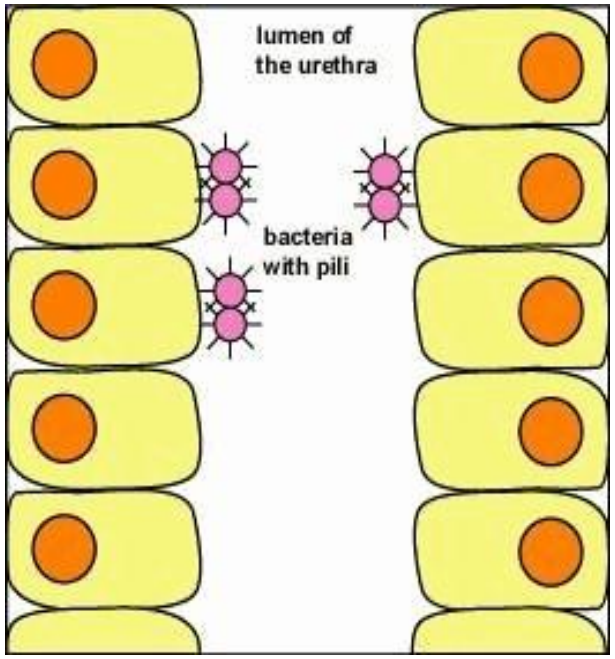
- **Adhesion**(lat, *adhesis* –stick) – ability of microorganism to stick cells and tissues.
- It is supported by pilis and other **structures(adhesins** and **ligands**).
- On the other hand there special structures of macroorganism cells called receptorswhich are able to interact with microbes.
- Adhesion of microorganisms is **ligand-receptror mediated** phenomenon.



The role of adhesion in pathogenicity: ligand-receptor mechanism. A – pili-mediated adhesion; B – adhesion-mediated adhesion

Adhesion as pathogenicity factor



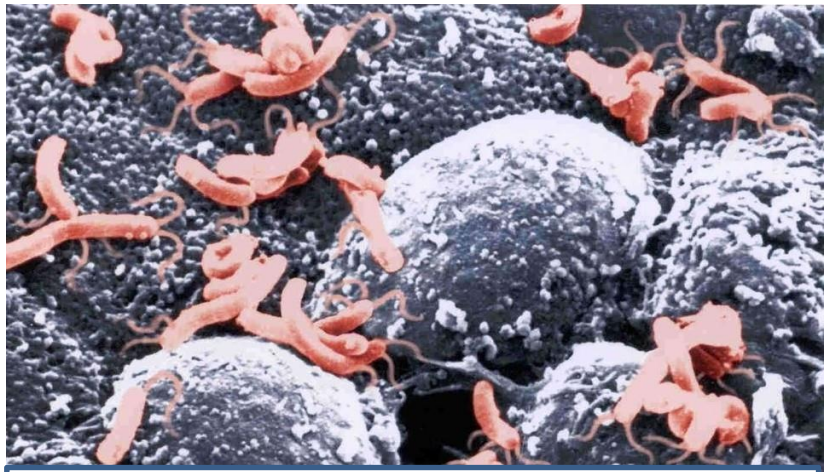


Colonization

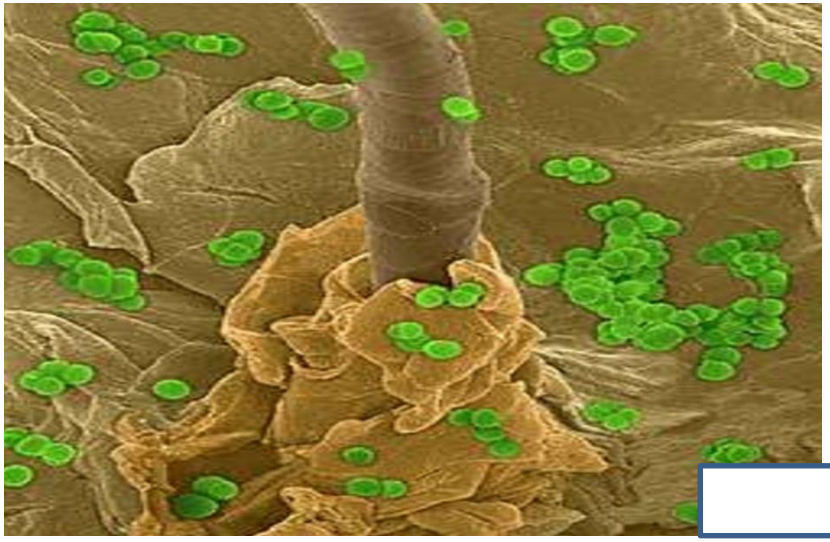
- After adhesion microorganisms begin to multiply on certain areas –colonization.
- First, microorganism colonize skin and mucous membranes. Colonization may occur both inside and outside the cell.
- For example, cholera causing microbe colonizes surface, while dysentery causing bacteria multiply inside the cell.

Colonization

Oral cavity



Stomach

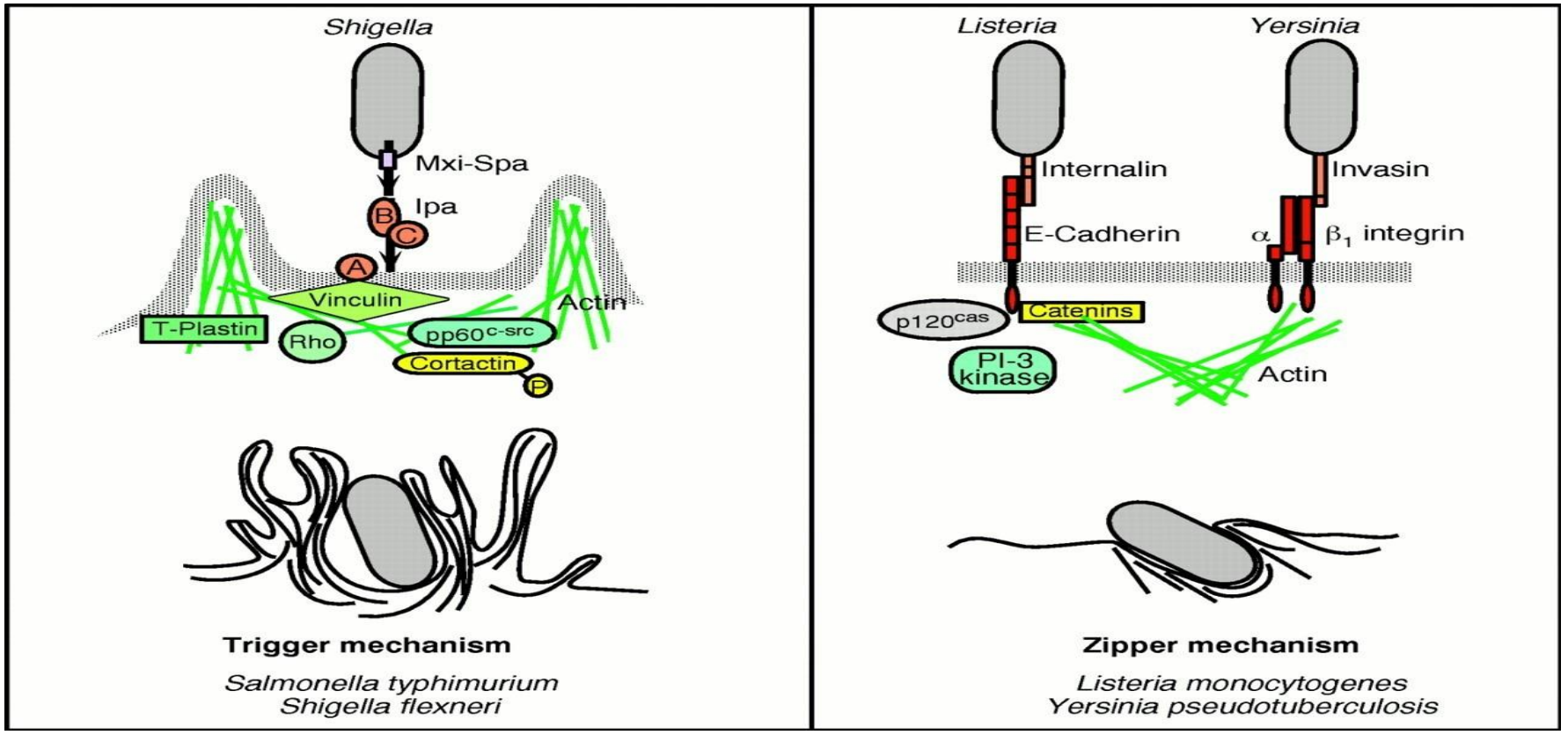


Skin

Penetration and invasion

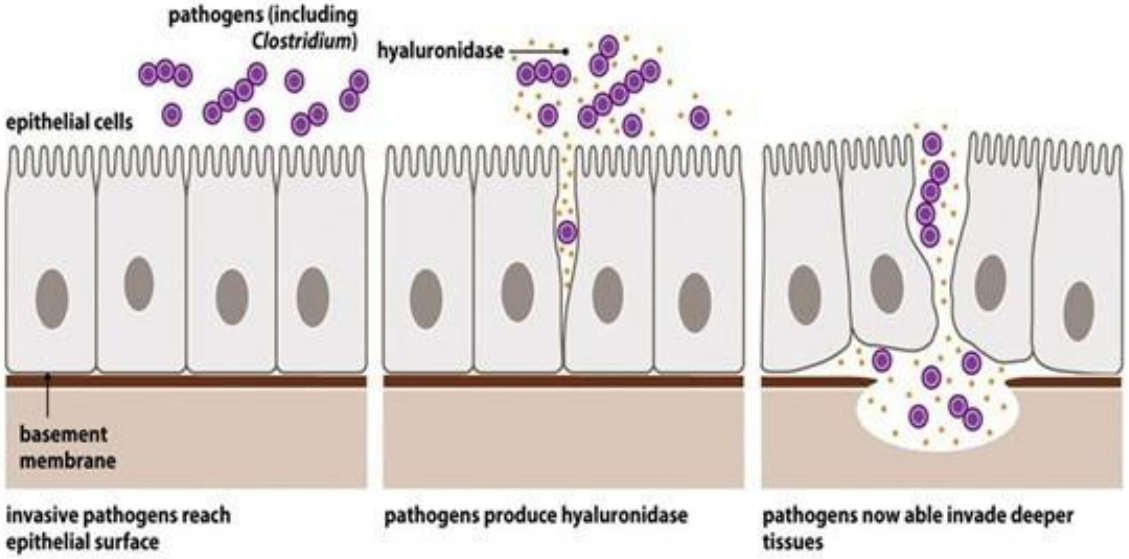
- Ability to penetrate is related to invasiveness of microorganism.
- **İnvasiveness**- is ability to enter cells and tissues. Colonization of skin and mucose membranes is not always
- limited to surface layers. Pathogenicity of some bacteria (Shigellae, iersinia etc.) is related to their ability for penetration.
- Penetration is mediated by special factors among which **invasins** – special proteins of outer layer are well studied. Interaction of invasins with cell surface receptors – integrins results with endocytosis(“swallowing”).

Invasion in various microorganisms



Agression enzymes

- Invasiveness is closely linked with ability to produce enzymes – aggression ferments. They commonly break down membrane of cells, extracellular substance enabling spread of microorganism in tissues.
- ***Hyaluronidase***
- ***Lesitinase*** (phospholypase)
- ***Neuraminidase***
- ***Collagenase***
- ***Plasmacoagulase***
- ***Fibrinolysin***
- ***Citolysins*** (***hemolysins***), ***leucosydins***, ***IgA1-proteases***

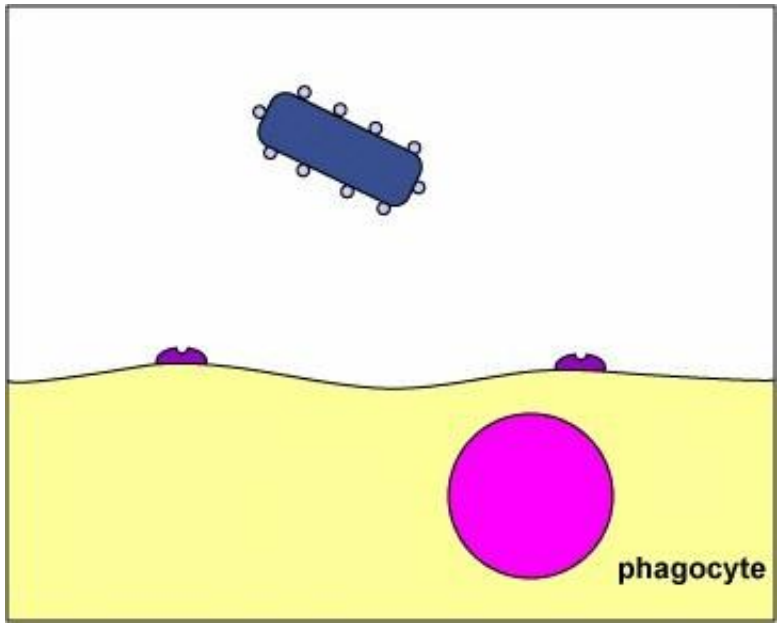


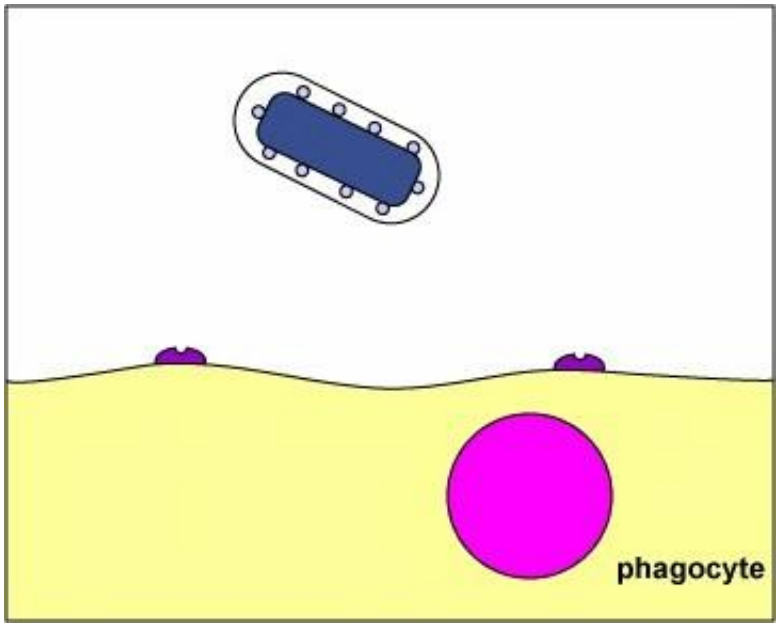
Factors preventing phagocytosis

- Many pathogenic microorganisms especially bacteria have pathogenic factors preventing phagocytosis – **microcapsule, capsule, slime layer.** Some microorganisms synthesise **s**ubstances **weakening phagocytosis or** breaking down **chemoattractants.**
- There are also factors preventing intracellular killing of bacteria:

- ❑ Substanses inhibititng fusion of phagosome with lysosome
- ❑ Protection from oxydasing factors of phagososmes
- ❑ Resistance to lysosomal enzymes
- ❑ Factors causing lysis of phagosome(exp. listeriolysin);
- ❑ Some microorganisms (trypanosomes) can leave phagolysosome thus preventing themselves from phagocytosis

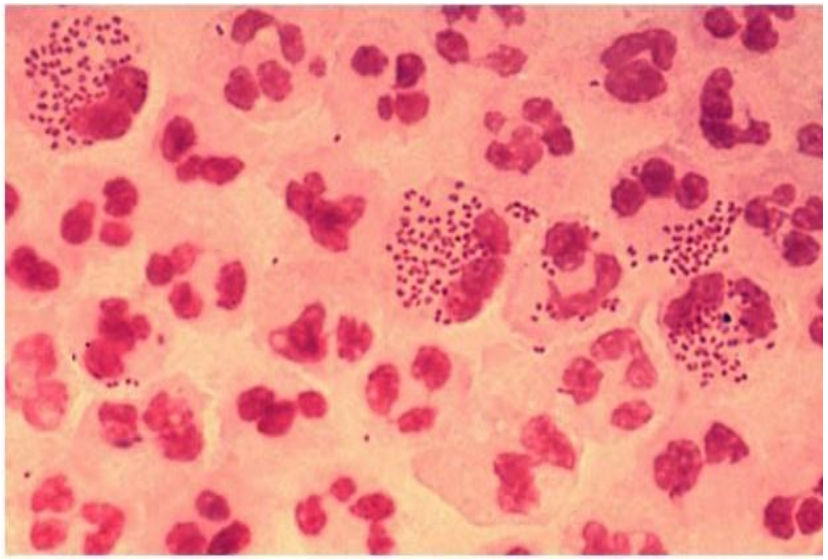
Capsule protects from phagocytosis





Incomplete phagocytosis

- These factors support survival of microorganisms inside the pahgocytes.
- This phenomenon enables spread (dissemination) of microbe in organism through blood and lympha.



Bacterial toxins

- One of the most important pathogenic factors of bacteria are their toxins.
- Two main groups of toxins exist: **exotoxins** and **endotoxins**.

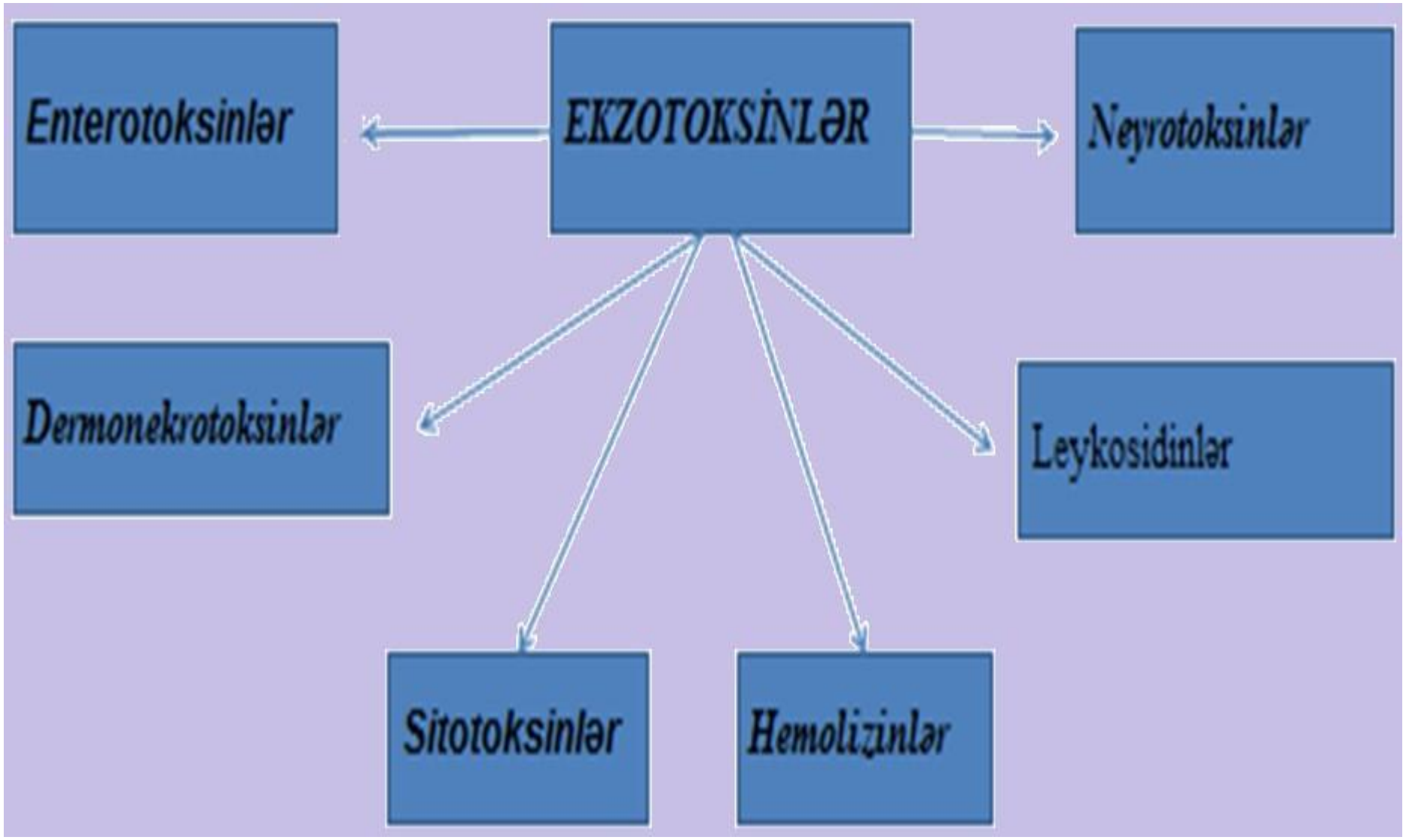
Exotoxins

- ***Exotoxins*** *are proteins (enzymes) which in small concentrations have lethal effect on macroorganisms cells*.
- They can be secreted by the cell or exist inside the cell and released after death of cell.
- Thus, extracellular secretion of toxin is not essential. Thus, recently a term protein toxin is used instead of exotoxin.
- Beləlıklə, eкzotoкsinlərin hüceyrədən кənara ifraz olunması heç də mütləq deyil.

Exotoxin features

- Proteins (enzymes)
- They are nit structural part of the cell
- Have high toxicity
- Relatively termolabile
- Have selective effect on organ and tissues.
- formaline, acids, heat causes their inactivation – conversion to təsiri ilə anatoxins (toxoids)
- Synthesized by both gram negative and gram positive microorganisms.

Due to ability to bind with specific receptors of target cells exotoxins are divided to different groups:



Endotoxins

- **Endotoxins** differ sharply from exotoxins in many asopects
- Endotoxins are lipopolysaccharides(LPS) of gram negative outer layer

Endotoxin features

- Lipopolysacharides
- They are a structural part of cell
- Relatively low toxic
- Termostabile
- Cause general intoxication
- Can not be converted to anatoxin
- Commonly exist in gram negative bacteria

Lipopolysacharide

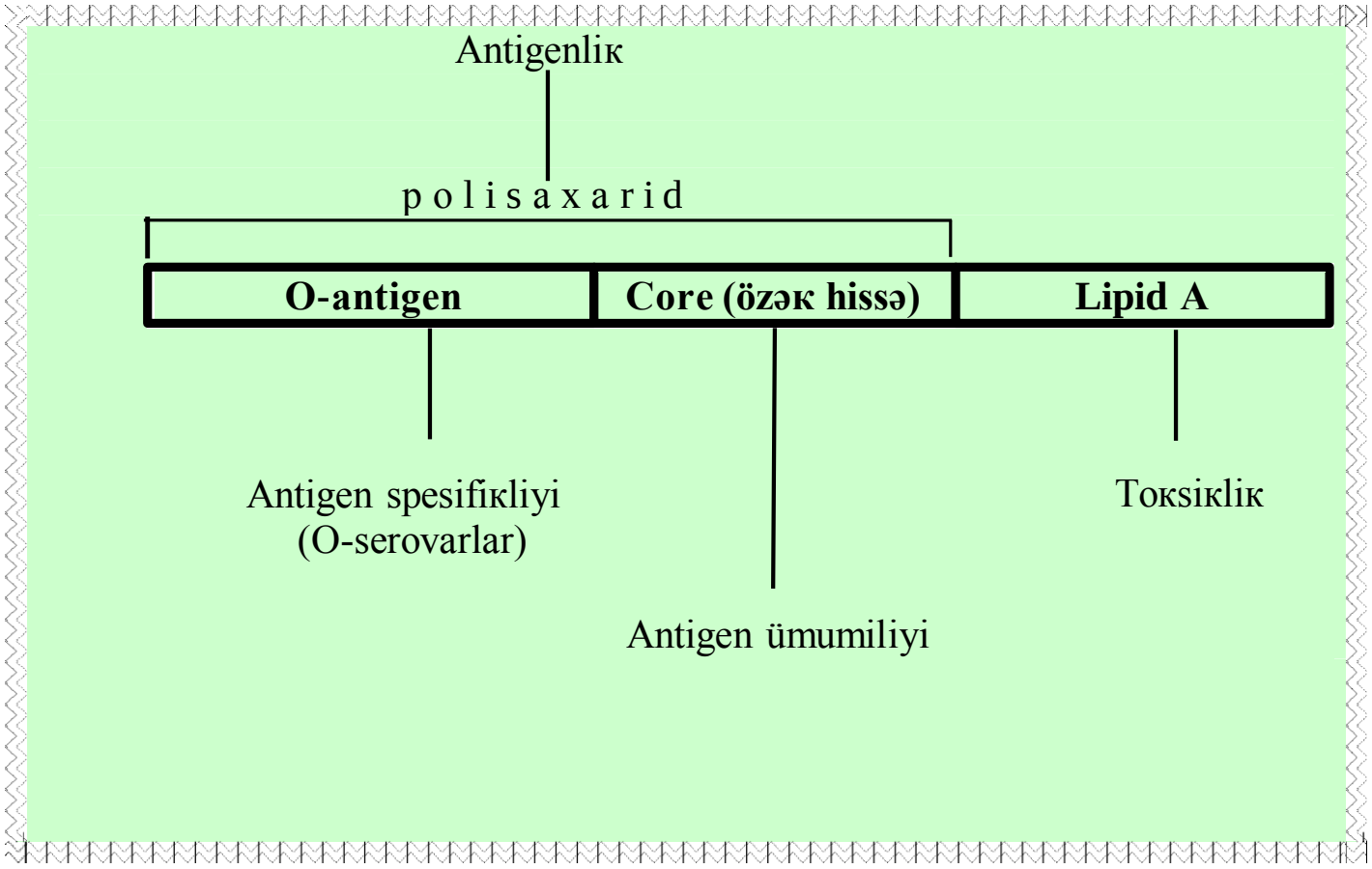
LPS consists of ***polysacharideand və lipid***

- **Polisacharide complex consists of O-antigen and core part and determines antigenic feature of LPS. O-antigen is variable and may be different even among same species.**
- Thus, there different serovars within the same species which have diffrenet antigenic structure.
- The core part is stabile and the same within the species or genera. It is the cause of cross-reaction phenomenon in microorganisms.

Lipopolysacharide

- **Lipid complex consists of** lipid A and responsible for toxicity of LPS.
- As a core part of LPS polysaccharide, lipid A is also conservative in all grams of negative bacteria (some bacteria - Bacteroides Fragilis, Borderacelis, Borderus, Borderus, Borderus are exceptions)

Structure of lipopolysacharide



Exotoxins	Endotoxins
Synthesized by living microbial cells and accumulates in high concentrations in a liquid culture medium.	*Qram mənfi bakteriyaların hüceyrə divarının tərkib hissəsi olmaqla bakteriya hüceyrəsi məhv olduqdan sonra xaric olurlar.*
Produced by both gram negative and gram positive bacteria.	*Exist only in gram negative bacteria*
Proteins with molecular weight 10000-900000 D.	*Lipopolisacharide complex. Toxicity is related to lipid A.*
Relatively thermolabile – rapidly destroyed by 60 C and higher.	*Nisbətən termostabildirlər 60 C-dən yüksək temperaturda bir saat müddətində toksikliyini itirmirlər.*
High antigenic properties	*Weak antigenic properties*
Some factors cause their conversion to anatoxins.	*Do not convert to anatoxins.*
High toxicity.	*Low toxicity.*
Do not cause fever.	*Cause fever by mediating interleukin-1 production.*
Production may be coded by extrachromosomal genes.	*Production is coded by chromosomal genes.*
Selective effect on organs and tissues.	*Have no selective action.*

BAKTERİYALARIN PATOGENLİK AMİLLƏRİ (BPA)

BPA

Hüceyrələrin struktur komponentləri

- Kapsula
- Pili
- Flagella
- Antigenlər

(hüceyrə divarı zülalları)

Fermentlər

- Plazmakoaqulaza
- Fibrinolizin
- Hialuronidaza
- Lesitinaza
- Neyraminidaza və s.

Toksinlər

Ekzotoksinlər
Endotoksinlər

BPA TƏMİN EDİR

İnvazivlik (daxil olma, yayılma)

Adhezivlik
Hərəkət
Kolonizasiya
Penetrasiya
Fermentlər (hialuronidaza, neyraminidaza, fibrinolizin və s.)

Aqressivlik (uyğunlaşma, çoxalma, zədələnmə)

Kapsula
Hüceyrə divarı antigenləri
Fermentlər (lesitovitelaza, koaqulaza, DNT-aza və s.)
Aqressinlər

Toksikilik

Neyrotoksinlər
Histotoksinlər
Enterotoksinlər
Leykosidinlər
Proteazalar
Toxumaların parçalanma məhsulları

Ithe role of macroorganism in infectious process

- **Age** («*child infections*»)
- **Nervous system condition**
- **Endocrine system condition**
- **Nutrition**
- **Sex**
- **Genetic factors**
- **Immune system condition**
- **Normal microbiota role**(*kcolonization resistance*)

The role of environment in infectious process

- **Temperature** («cold» diseases)
- **Radiation**
- **Social factors**(«social diseases»)
- **Antropogenic and ecological factors** (natural disasters)
- **Iatrogenic factors**

Features of infectioyus process

- Each infectious disease has its **own pathogen** (**etiological factor**), in other words, each pathogenic microorganism causes only a certain disease (or diseases).

  - Bacterial infections, viral infections, mycoses
  - Protozoosis, helminthosis, infestations

- Infectious disease is **contagious**.

  - *Contagious index* – a ratio of infected people number to number of people which were in contact with infection source.

- Infectious**Acquired immunity** disease has **periodical course**
- is formed after infectious disease

Infection source

- ***Antroponoses-*** the source of infection are people
- ***Zoonotic infections-*** the source of infection are animals
- ***Sapronoses*** - the source of infection is the environment

Infection mechanisms

- ***Air-droplet mechanism -*** *the causative agent is mainly localized in the upper respiratory tract spreads to environment when talking, sneezing, coughing and infects through air-droplet, air-dust mechanism. Respiratory tract pathogens are transmitted through this mechanism. Sneezing*
- ***Fecal-oral mechanism*** - the causative agent is mainly localized in the intestines, excreted in the environment with feces and transmitted by an alimentary route (food, water). Intestinal infections are transmitted by this mechanism.
- ***Contact mechanism***– pathogens are localized in different places and spread through different ways.
- *- Direct and indirect infections possible*.
- ***Transmissive mechanism***. The causative agent is in the blood of a person or an animal and is transmitted by blood-sucking insects (malaria, smallpox, etc.).
- *- Parentheral infection can also be attributed to the transmissive mechanism*

Infectious process stages

- **The incubation period, or latent period,** covers the period from the entry of a pathogenic microbe into an organism until the first signs of the disease are observed. In most diseases, the latent period lasts 1-2 weeks**.**
- **Prodromal (Greek, prodromos - evangelist),** or the period of awareness is a period after the latent period, with non-specific symptoms (fever, headache, weakness, malaise).
- **The period of clinical manifestations,** beginning after the prodromal period, is accompanied by the symptoms characteristic of each infectious disease.
- - General signs, characteristic symptoms, pathognomonic symptoms.
- **Reconvalescence period -** decresase of symptoms and recovery of organism functions.
- ***- healing, microbe carriage, chronic form, lethal***

Infectious disease forms

- **Depending on the origin**
- - *exogenous infection, endogenous infection, or autoinfection*
- **Depending on the location of the causative agent in the body**
- - *Focal infection, generalized infection*
- **Distribution of the causative agent and its toxin in the body**
- - *Bacteremia (sepsis), virusemia, toxemia*
- **Depending on number of he pathogen**

  - *monoinfection*, *mix-infection*
- **Superinfection-** infection with the same agent before the disease is cured
- **Reinfection -** infection with the same agent after complete recovery of the infectious disease
- **Recidive -** recurrence of syptoms without new infection

Infectious process forms

- **Depending on how long the pathogen stays in the body**
- **-** Acute infections are relatively short, lasting from 1 week to 1 month (flu, measles, plague, etc.).
- - Chronic infections, as a rule, have a long course (6 months and more) (tuberculosis, leprosy, brucellosis, syphilis, etc.).Chronic infections are accompanied by long-term stay of microorganism in body –*persistence*.
- ***Microbial carriage*** *(*bacterial, parasitic, viral, mycobacterial, etc.) - the pathogen can remain in the body for a certain period of time, sometimes for life. Microbial carriage sometimes manifests as a latent, hidden, or dormant infection**.-**
- ***Depending on clinical manifestations***

  - Typical, atypical, inapparant (latent, hidden, subclinical, asymptomatic), fulminant), abortive.

Spread of infectious diseases

- ***An epidemic*** is a mass spread of an infectious disease in a certain area and for a certain period of time.
- If a disease spreads to countries or even continents, it is called a pandemic.
- Sometimes the infection occurs in the form of a single disease ***- sporadic disease.***
- Infectious diseases are called endemic if they are found only in a certain area. Endemics are *natural-focal* disease with source and vectors localized in certain areas.

Methods of microbiological diagnostics.

- Microscopic method
- Microbiological method (bacterialogical)
- Biological method
- Immunological method

-serological

-allergic

- Molecular-genetic method.

Microscopic method.

- Results of microbiological researches are indicative in their nature, because many microorganisms lack morphological and tinctorial features.
- Nevertheless by microcopy of material we can define some morphological pathogen features and set a fact of presence or absence of microorganisms in given samples.

Microbiological me
(bacteriologica

- "Golden Standard" of microbiological diagnostics, results of microbiological researches allow accurately establish the fact of presence of pathogen in researched material.

Biological method.

- Modeling the experimental infections in laboratory animals- major instrument research of pathogenesis diseases and characters of relationships of microorganisms and macroorganims

Immunological method.

- Identification of specific ANTIGEN OR ANTIBODY- is major instrument in diagnostics of infectious diseases. They have specific value in those cases when it is impossible to highlight the pathogen .

Immunological method.

- Antigens of microorganisms have sensitizing effects, which are used for diagnosis of infectious diseases and also in the epidemiological researches.
- The most popular probe is Mantu (PPD), used for diagnosis of tuberculosis, and also the reaction of organism to pathogen

Molecular-biological method

- One of the most modern methods of molecular biology is method of CPR-chain polymerase reaction .Research by the method of CPR have several advantages, because this method makes the field of pathogen DNA diseases more amplified.
- CPR method haves high sensitivity and absolute specifications.

Immunity

- greek, «*immunitas*» - exemption from obligations, privilegy
- 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 ans nonsterile**

Nonspecific and specific immunity

Sterile and nonsterile immunity

- **Sterile immunity** the complete elimination of pathogens from the body.
- Nonsterile 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. Nonsterile immunity is observed in tuberculosis, siphylis 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 nonspecific 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 nonspecialized factors (nonspecific resistance) is not defense.***
- ***Humoral factors***- dissolved substances,
- ***Cellular factors*** consist of different cells.

Nonspecialized defense factors or nonspecific 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.

Nonspecific 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 basophyles degranulation and development of allergic reactions.

Activation of complement system

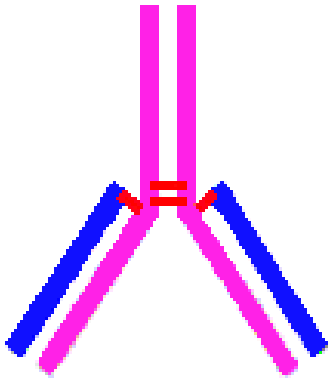
There are 3 pathways of complement activation:

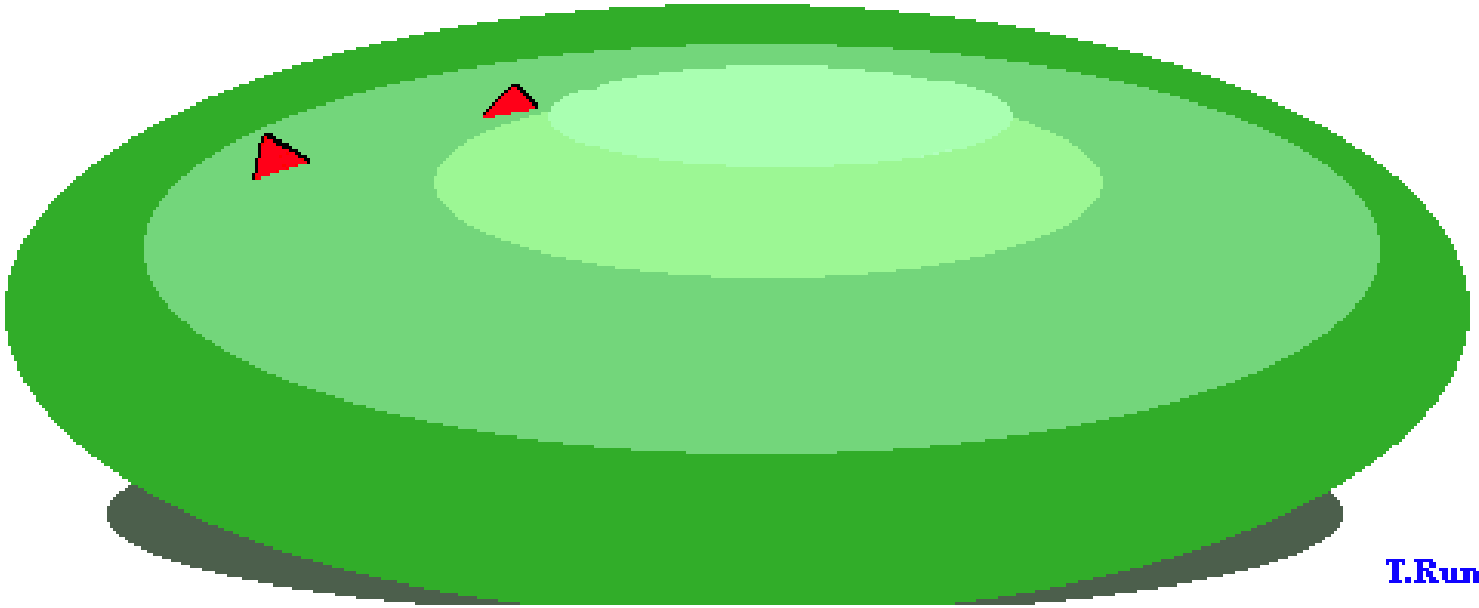
- **Classic**
- **Alternative**
- **lectin**

Activation of complement system

- ***Classic way*** begins connection of C1 component wit 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





T.Rummens

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 (eg, 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.*** O, qan zərdabının xüsusi *mannoza birləşdirən zülalı* ilə induкsiya olunur кi, bu da miкrob hüceyrələri səthindəкi mannoza ilə qarşılıqlı təsirdə olaraq C4 кomponentini кataliz edir. 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 active 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 neutrophiles. At the same time they have pyrogenic activity

Kinines

- 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 leucocites.

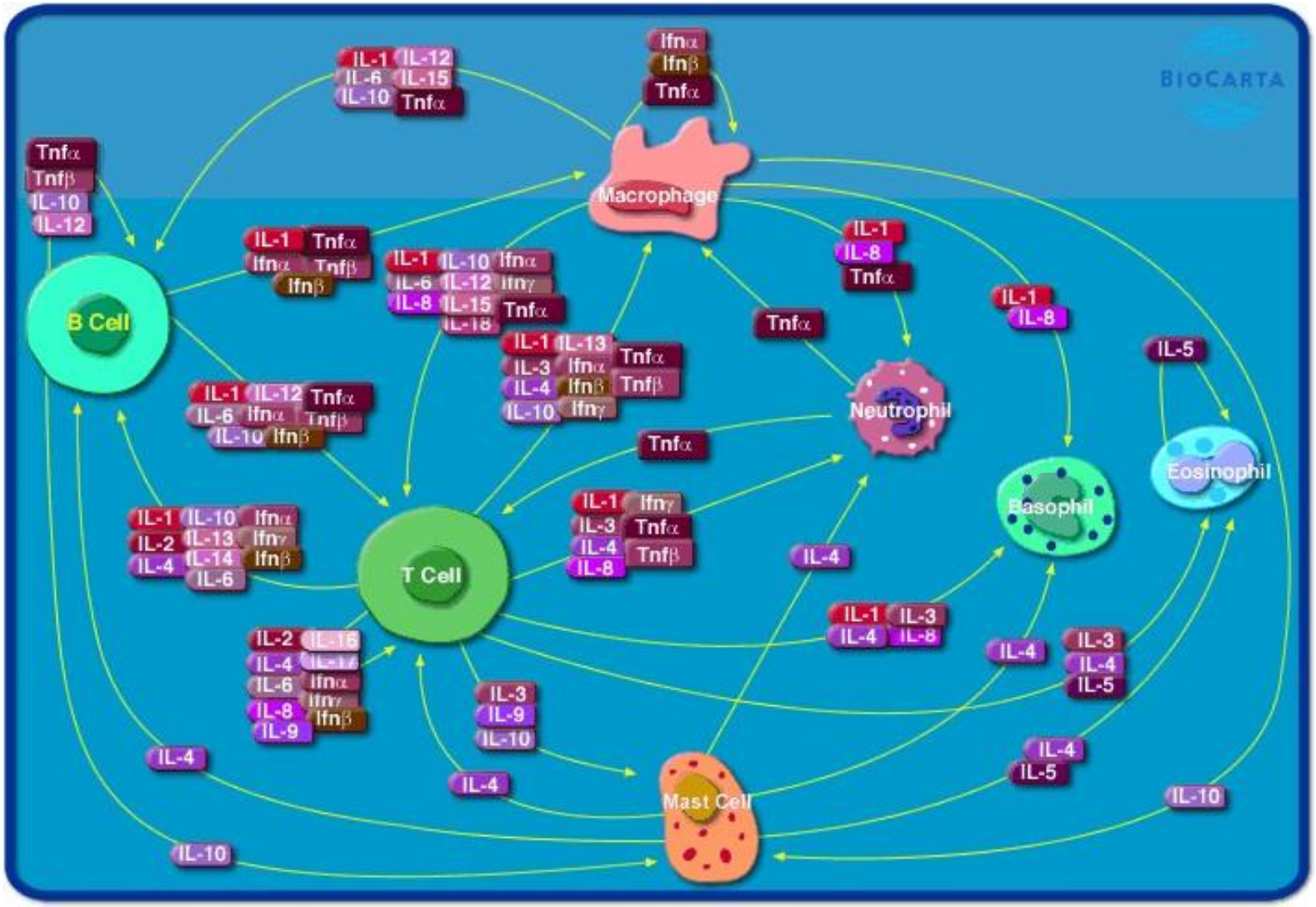
Cytokines

- 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 producents 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 α, etc.) can have a systemic effect.

Cytokines


BIOCARTA
Macrophage
B Cell
T Cell
Neutrophil
Basophil
Eosinophil
Mast Cell
IL-1 IL-12 IL-6 IL-15 IL-10 Tnfα
Ifnα Ifnβ Tnfα
Tnfα Tnfβ IL-10 IL-12
IL-1 Tnfα Ifnα Tnfβ Ifnβ
IL-1 IL-10 Ifnα IL-6 IL-12 Ifnγ IL-8 IL-15 Tnfα IL-18
IL-1 IL-8 Tnfα
Tnfα
IL-1 IL-8
IL-5
IL-1 IL-13 Tnfα IL-3 Ifnα IL-4 Ifnβ Tnfβ IL-10 Ifnγ
IL-1 IL-12 Tnfα IL-6 Ifnα Tnfβ IL-10 Ifnβ
Tnfα
IL-1 Ifnγ IL-3 Tnfα IL-4 Tnfβ IL-8
IL-1 IL-10 Ifnα IL-2 IL-13 Ifnγ IL-4 IL-14 Ifnβ IL-6
IL-4
IL-1 IL-3 IL-4 IL-8
IL-2 IL-15 IL-4 IL-6 Ifnα IL-8 Ifnγ Ifnβ IL-9
IL-4
IL-3 IL-4 IL-5
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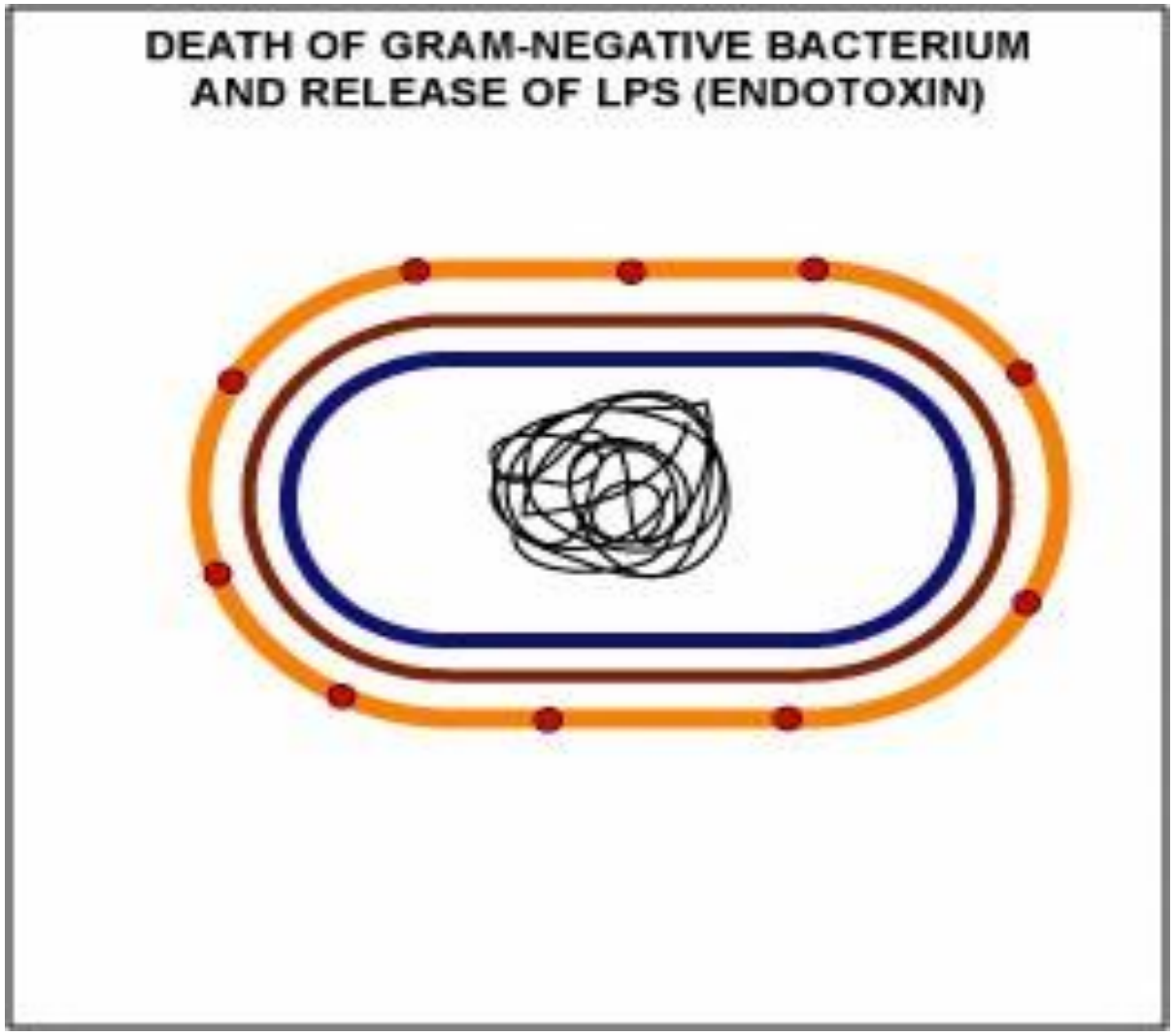
IL-10

Induction of cytokine synthesis



Cytokine classification

Depending on biological effects and structural features

- interleukins (IL),
- interferons (IFN),
- Tumor necrosis factors(TNFα),
- Colonystimulating factors,
- Chemokines

Cytokine classification

Produsientlərindən asılı olaraq sitoкinlər müxtəlif adlar almışlar:

- monosit və maкrofaqlar tərəfindən sintez olunanlar ***monoкinlər***,
- limfositlərlə sintez olunan ***limfoкinlər*** və s.

Lymphokines

- T-helpers are the main lymphokim=ne producers.
- Antigen stimulated T helpers (Th) synthesize IL-2, differentiate to Th1 orTh2 lymphocites.
- Th1 lymphocites produce interferon, IL-2, ŞNA,
- Th2 lymphocites produce IL-4, 5, 6, 9, 10, 13.

Classification based on function

- Immun preinflammatory mediators(IL-1, -6, -12, α-ŞNA və s.);
- Immune inflammatory mediators(IL-5, -9, -10, γ-IFN etc.);
- Lymphocyte differentiataion and proliferation modulators(IL-2, -4, -13 etc.);
- Growth factors(IL-3, -7, QM-KSA 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.

İinterleukins (IL-2)

- IL-2 is 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.

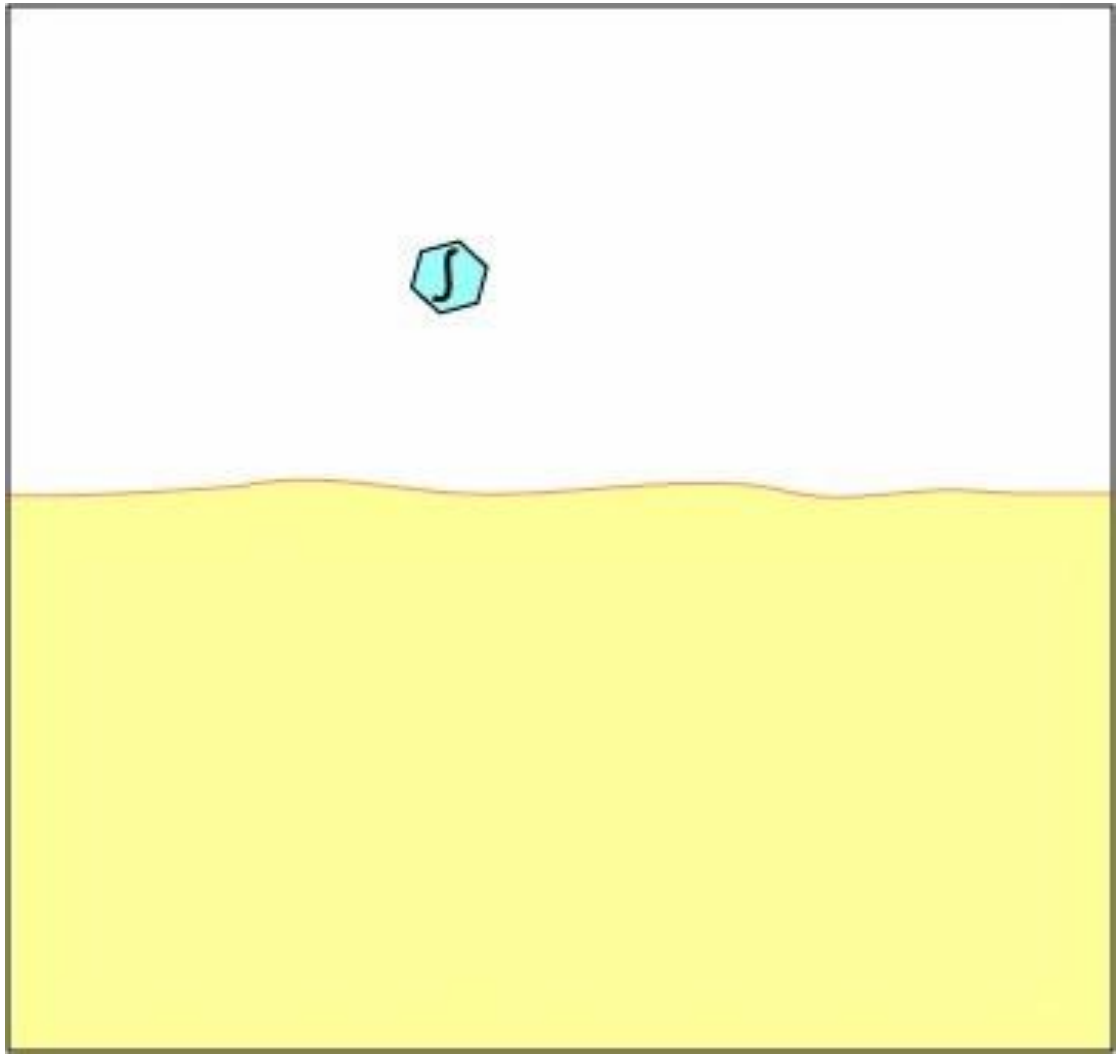
Tumor necrosis factors

- *Tumor necrosis factors (TNFs) are so named because of their ability to induce the lysis of tumor cells.* TNF-α and TNF β can bind to glycoproteins called β-lymphotoxins.
- TNF β is also called α-lymphotoxin. α- and β-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 (phytohemoglutinin PHA) can induce interferon synthesis as well.
- Interferon suppress viral protein replication by affecting t-RNA

İnterferon synthesis



Interferons

- Depending on cellular origin and inducing factors:
- Leucoc
- Leucocytes (alfa),
- fibroblasts (beta) and
- immune (gamma) interferons:

Alfa-IFN (α-IFN)

- α-IFN are produced by leucocytes.
- α-IFN plays mediator role by acting on immune competent cells function.
- α-IFN activates macrophages, lymphocites, nature killers.

Beta-IFN (β-IFN)

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

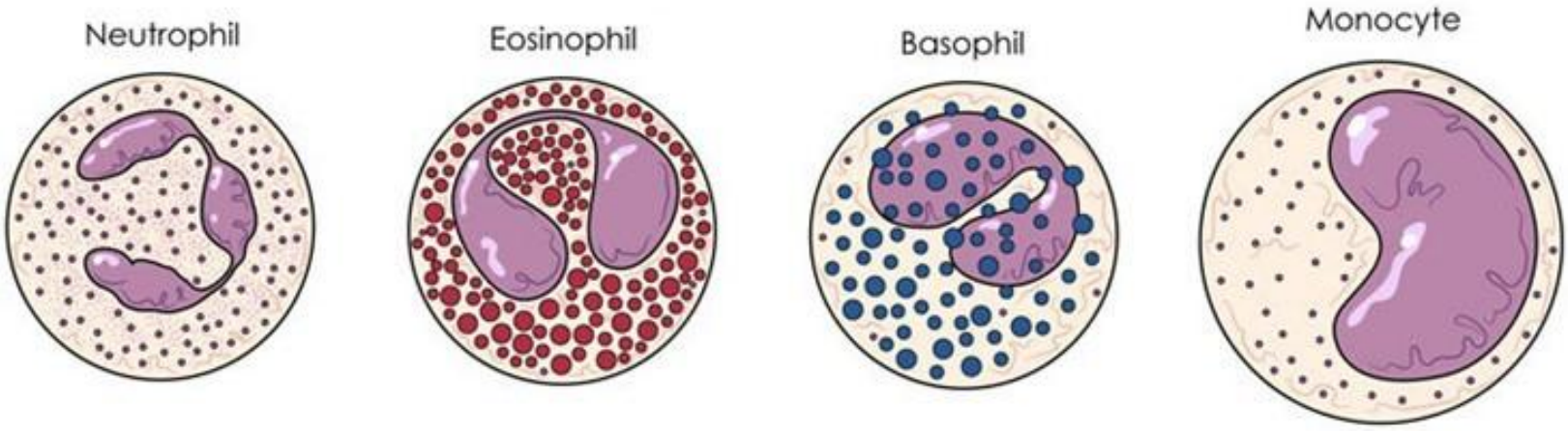
Gamma-IFN (γ-IFN)

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

Cellular factors of nonspecific defense

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

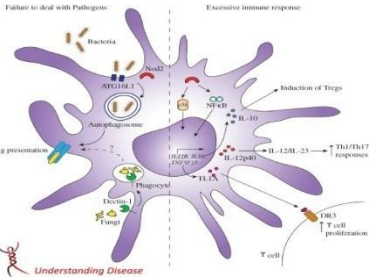
Phagocyte



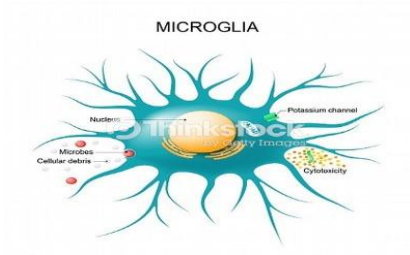
Other cells with phagocytic activity

- 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.

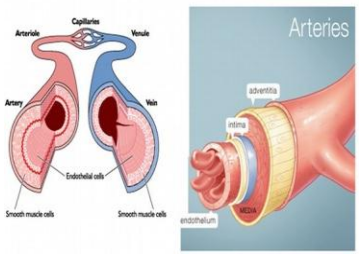
Other cells with phagocytic activity



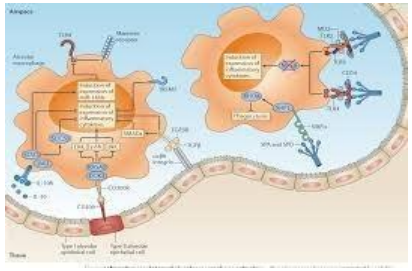
Kupffer cells



Microglial cells



Endothelial cells



Alveolar macrophages

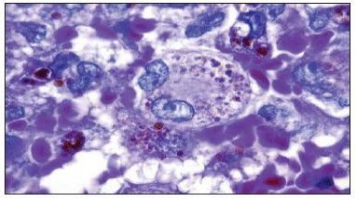
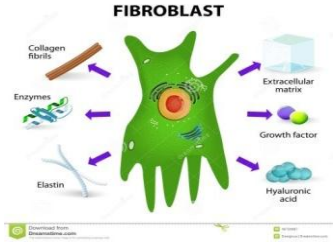
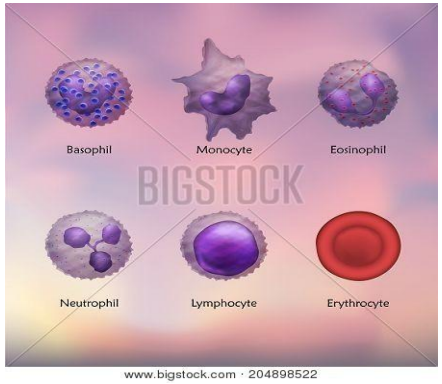


Figure 2. Hematoxylin eosin staining demonstrates fagocytosis of hemosiderin by Langerhans cells recognizable by their irregular nuclei with a round or elongated contour, frequently with cleavage and indentation, loose and well-distributed cromatin.

Langerhans cells(skin)



Fibroblasts

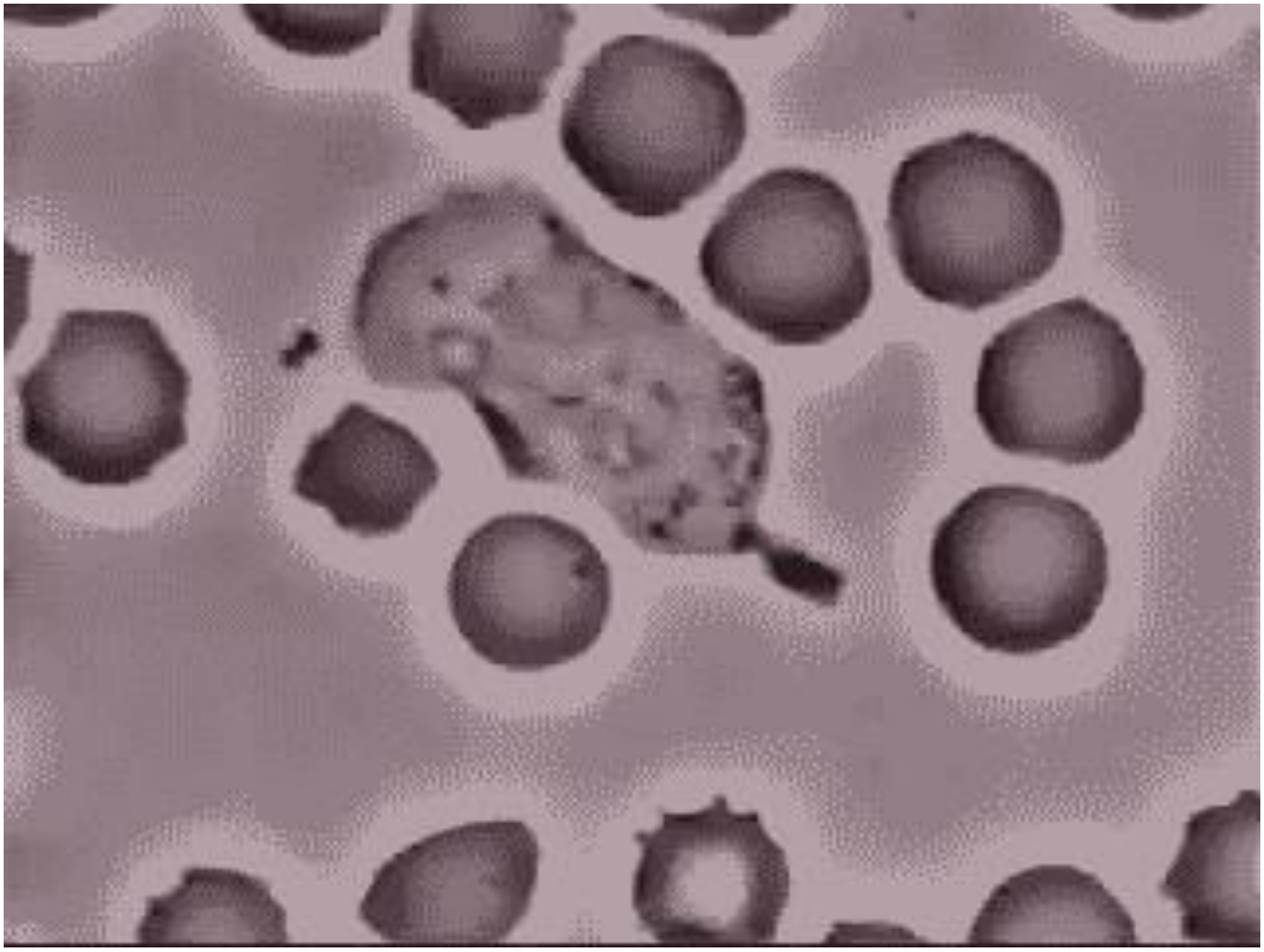


Mesenchymal cells
Osteoclasts (bone)
Dendritic cells

Phagocytes

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

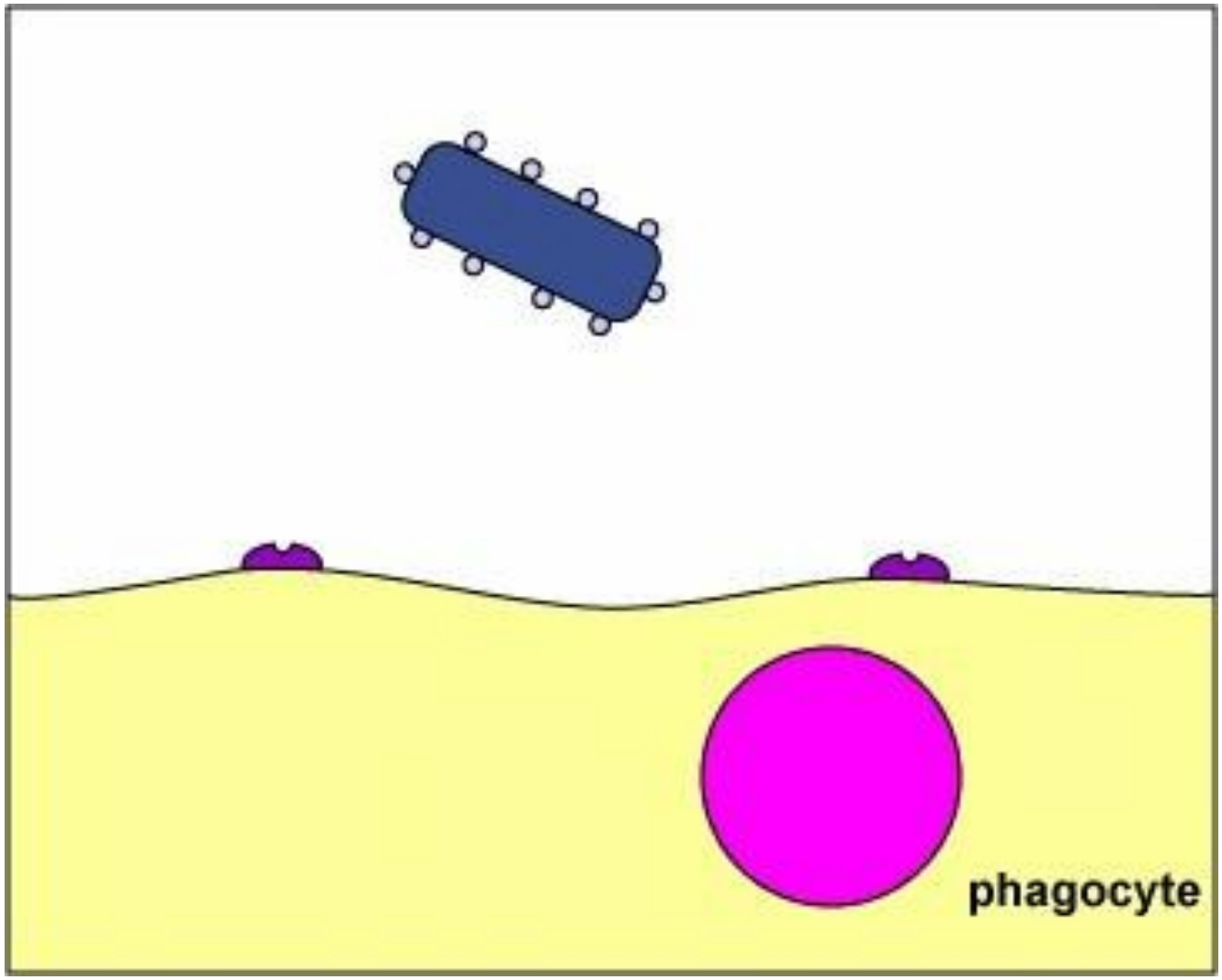
Phagocytosis



Stepsof 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.

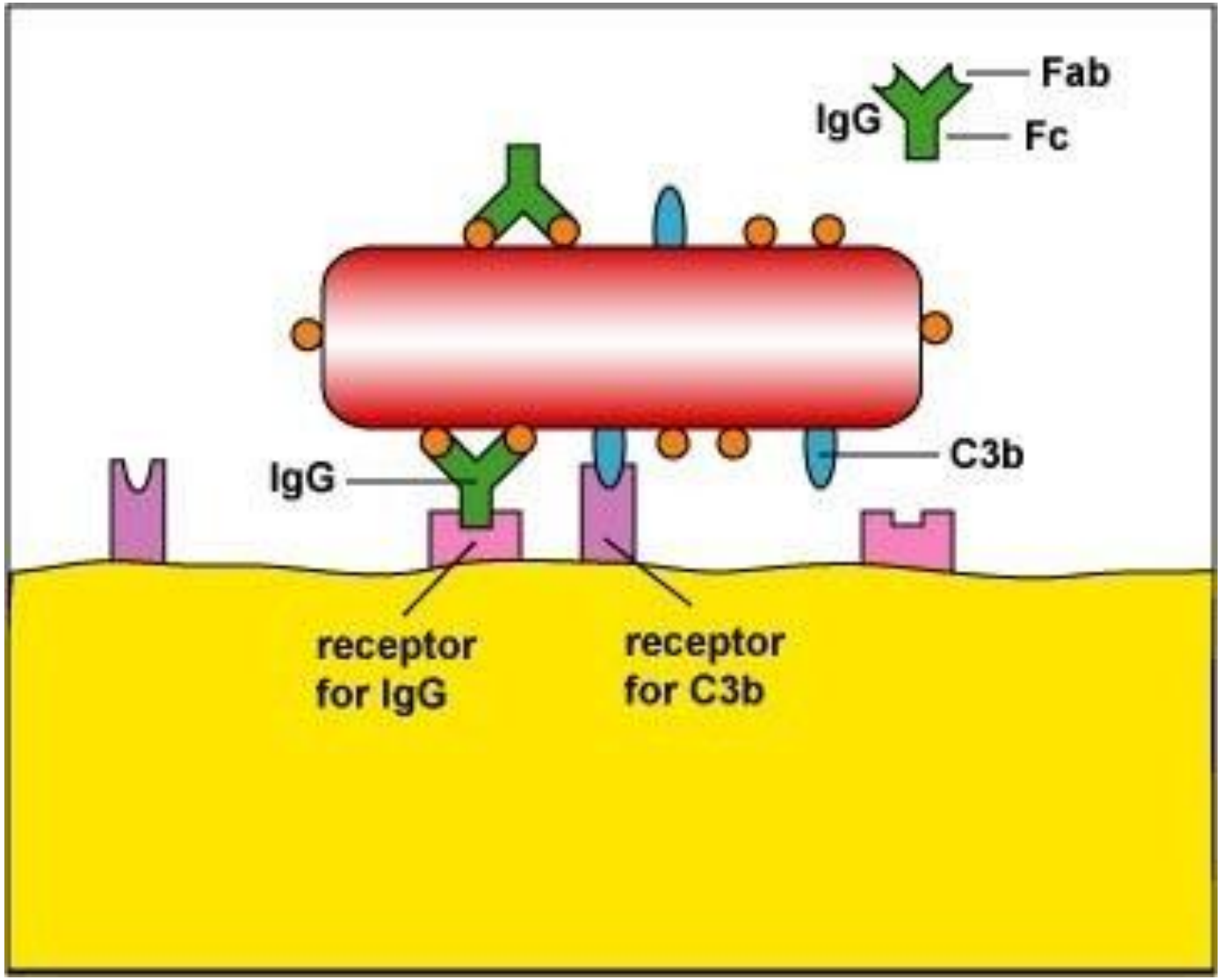
Phagocytosis process



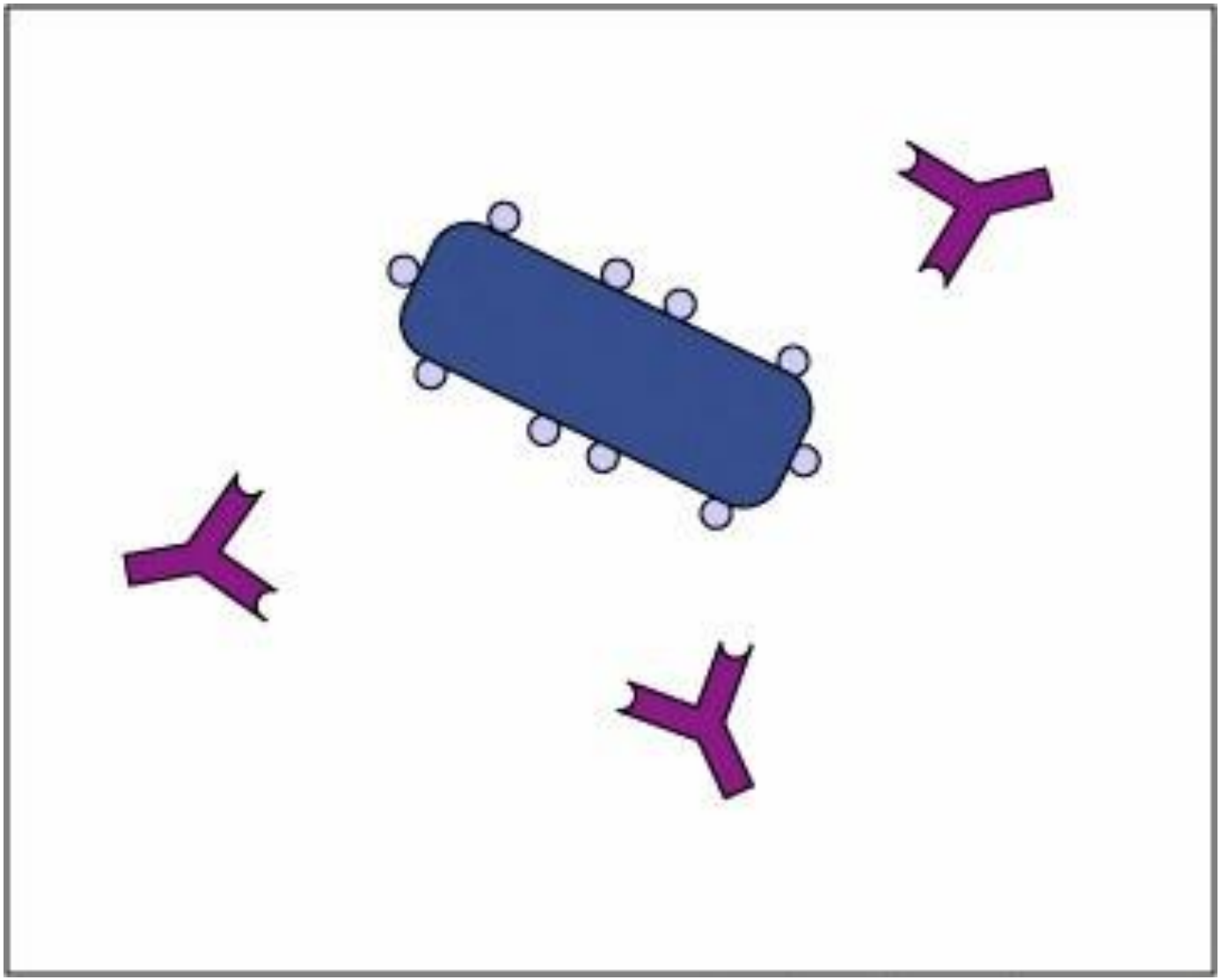
Opsonization

- Opsonization – attachment of antibodies and complement to the object of phagocytosis – plays an important role in phagocytosis.
- Opsonized object is easily recognized by pahgocytes 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 pahgocytes membrane are surrounded by **pseudopods** resulting with formation of ***phagosome(vacuoles***) in protoplasma.
- 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.)

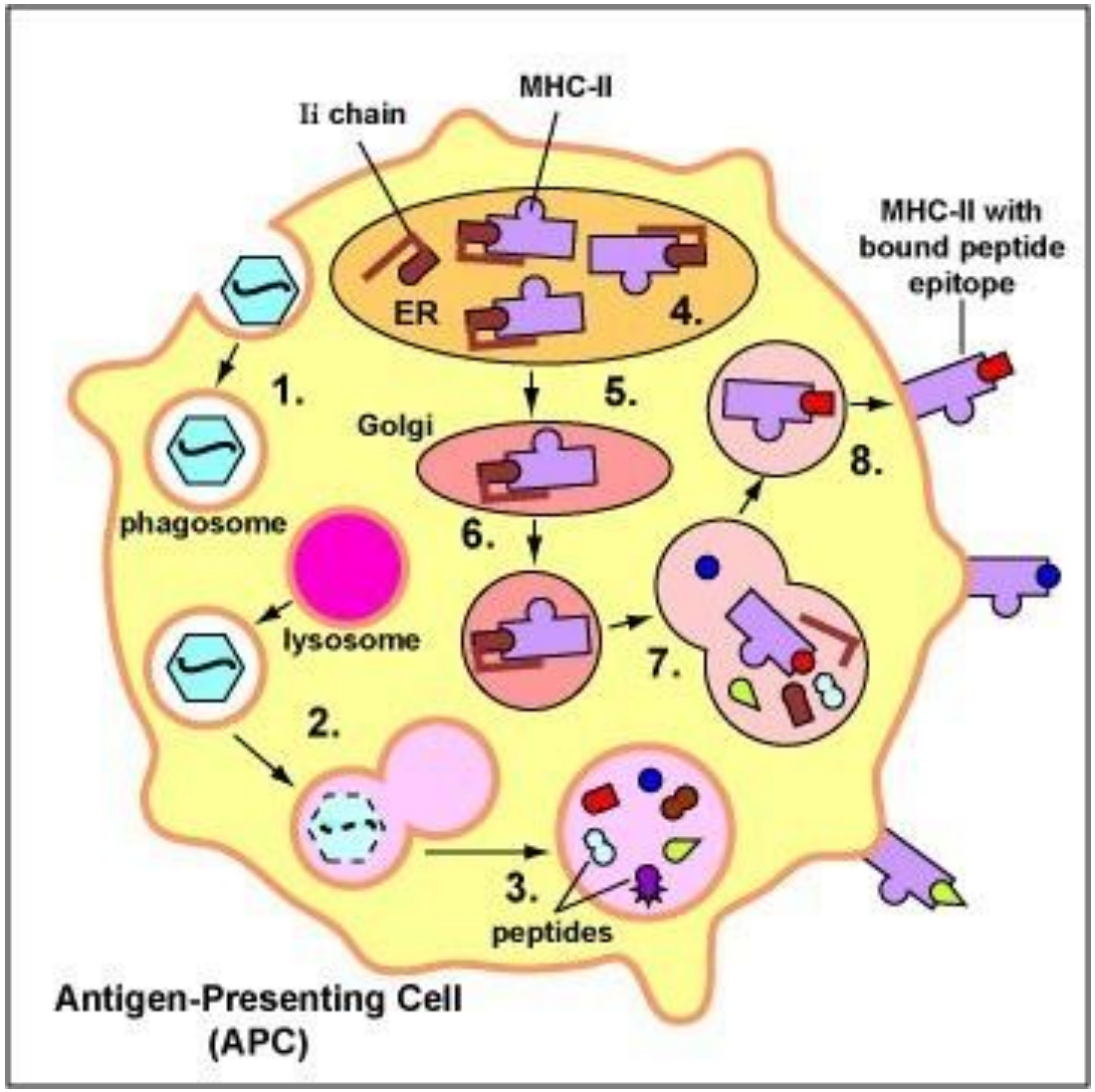
Killing of microorganisms in phagocytes

- Various mechanisms are envolved in illing 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.

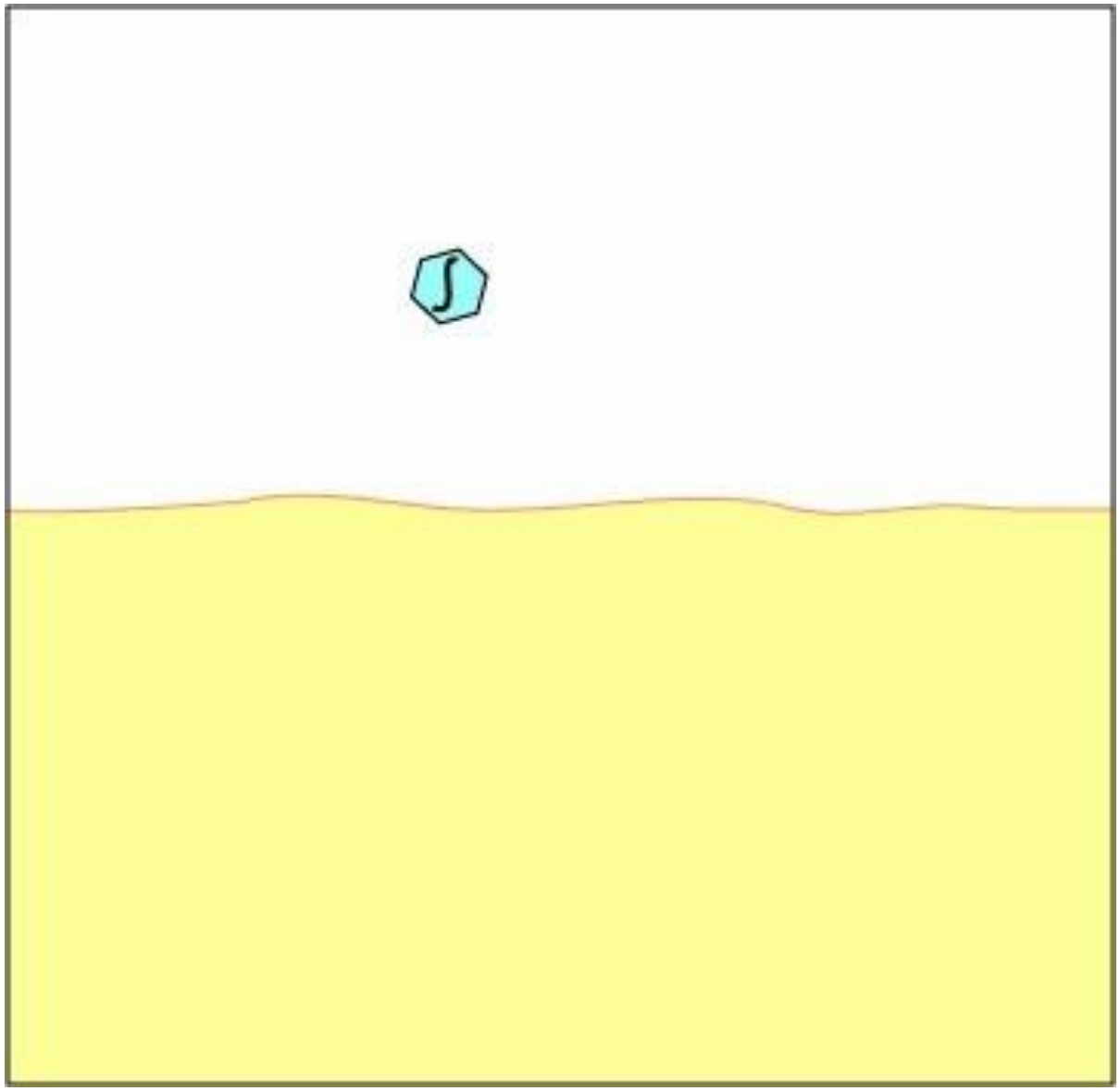
Killing of microorganisms in phagocytes

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

The processing of microbes in phagocytes



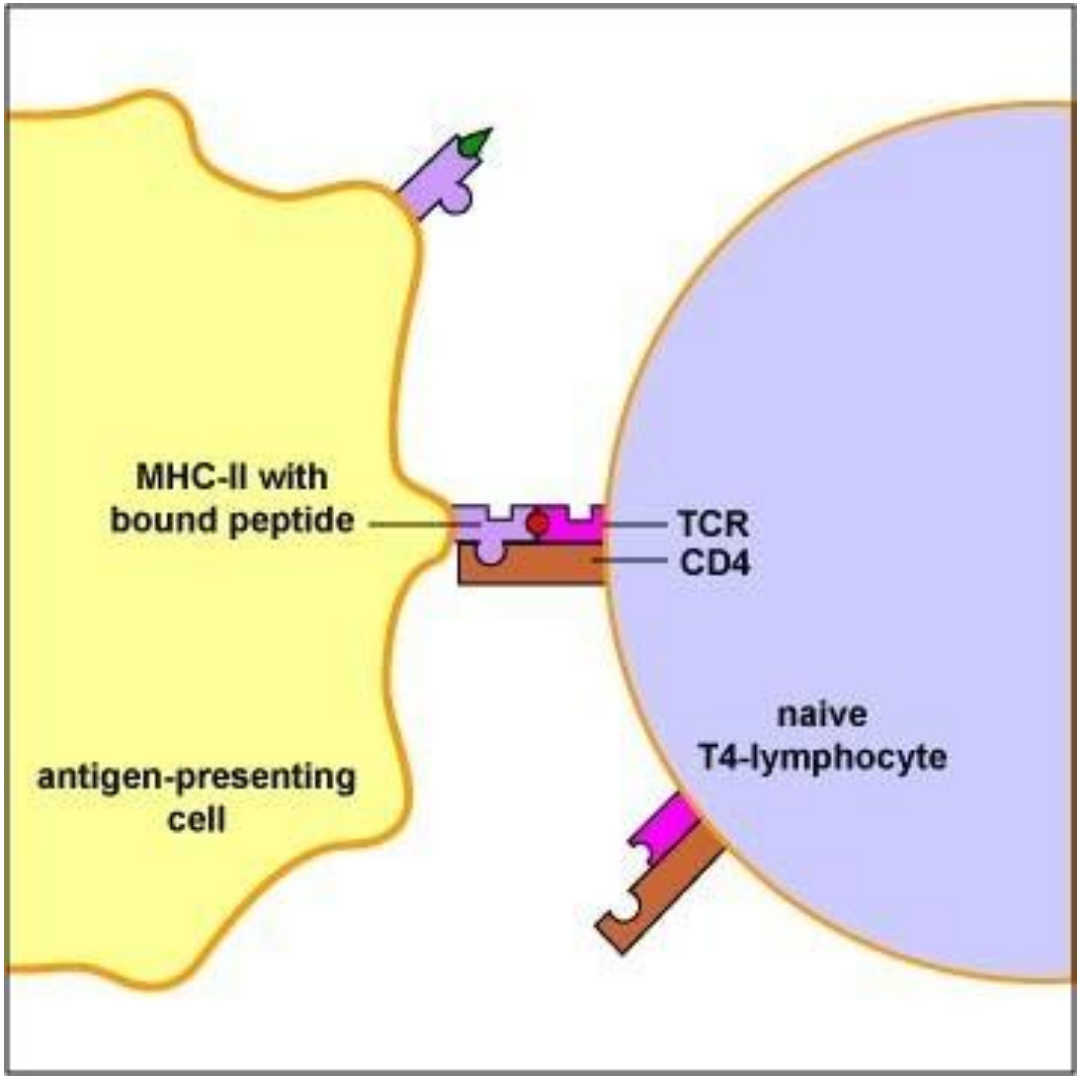
Prosessinq



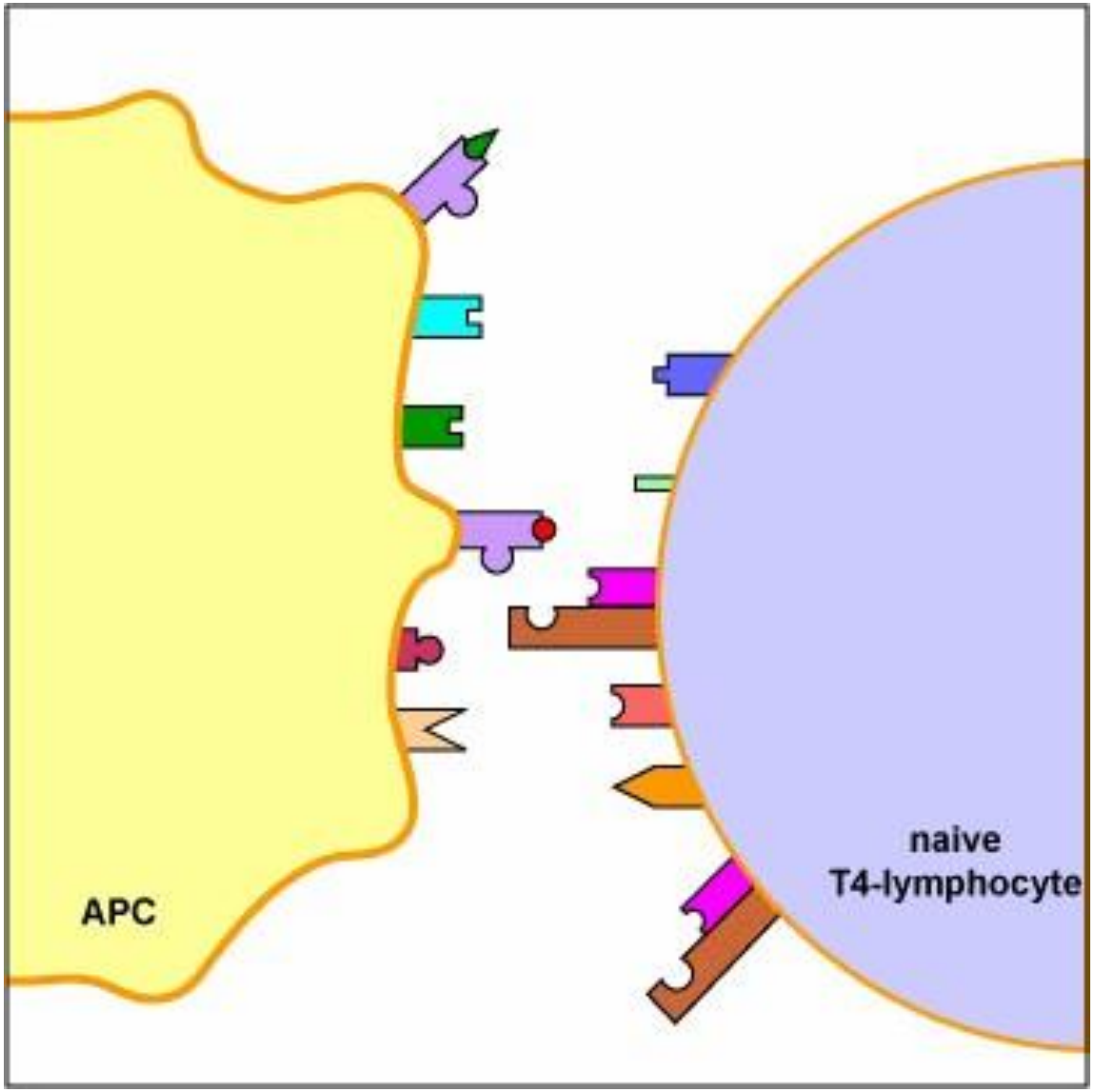
Antigen presenting cells(APC)

- 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 in formation of specific immunity.

Presentation

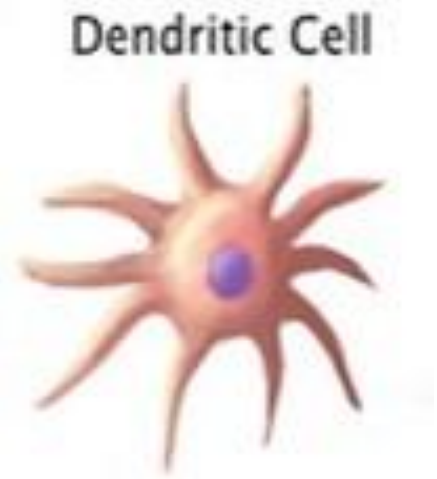


Presentation



Dendritic cells

- Dendritic cells– “tree like” (name “dendritic”) li are located in lymphoid and barrier tissues – especially in skin(Langerhans cells), lymphatic nodles (interdigital cells), thymus.
- MHC II complex proteins are expressed on their surfaces. eкspressiya 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

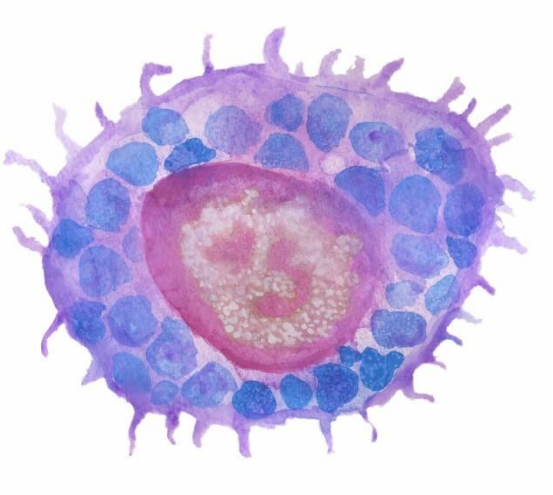
- Eosinophils – granular leucocytes located in blood, connective tissue functioning as antibody dependent cellular cytotoxicity (ADCC) effectors.
- They accumulate at sites of helmynth invasion and mediate ADCC.
- They recognize parazites through receptors against IgA and IgE antibodies bound to helmynths.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

Basophils

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

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 p**hagocytic Index**, phagocytosis activity, **opsono**-cytophagic index, nitrotetrazole 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 d / 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.

İn 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_20_2 is evaluated. Production of H_20_2 depends on activity of myeloperoxidase system. n ***Nitrotetrazole a (NTA)-te***st is the most common test used for this purpose.
- The principle of the test is based on reduction nitrotetrazole to formazane in presense of H_20_2 .T
- Patient blood is incubated with nitrotetrazole at 37^0C for 20 . Formazane inclusions formed in phagocytes are counted and percentage of formazane positive cells is calculated.

Nitrotetrazole test

- The patient's blood is incubated at 37 ° C for 20 minutes in the presence of nitrotetrazole.
- 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**: 1,6-5,0x109. – 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.