LECTURE IX

Immunoglobulins (antibodies) and their classes.

Immune response reactions, their types and mechanisms (production of antibodies, immune phagocytosis, hypersensitivity reactions, immunological memory, immunological tolerance, antibody-dependent and independent cytotoxicity).

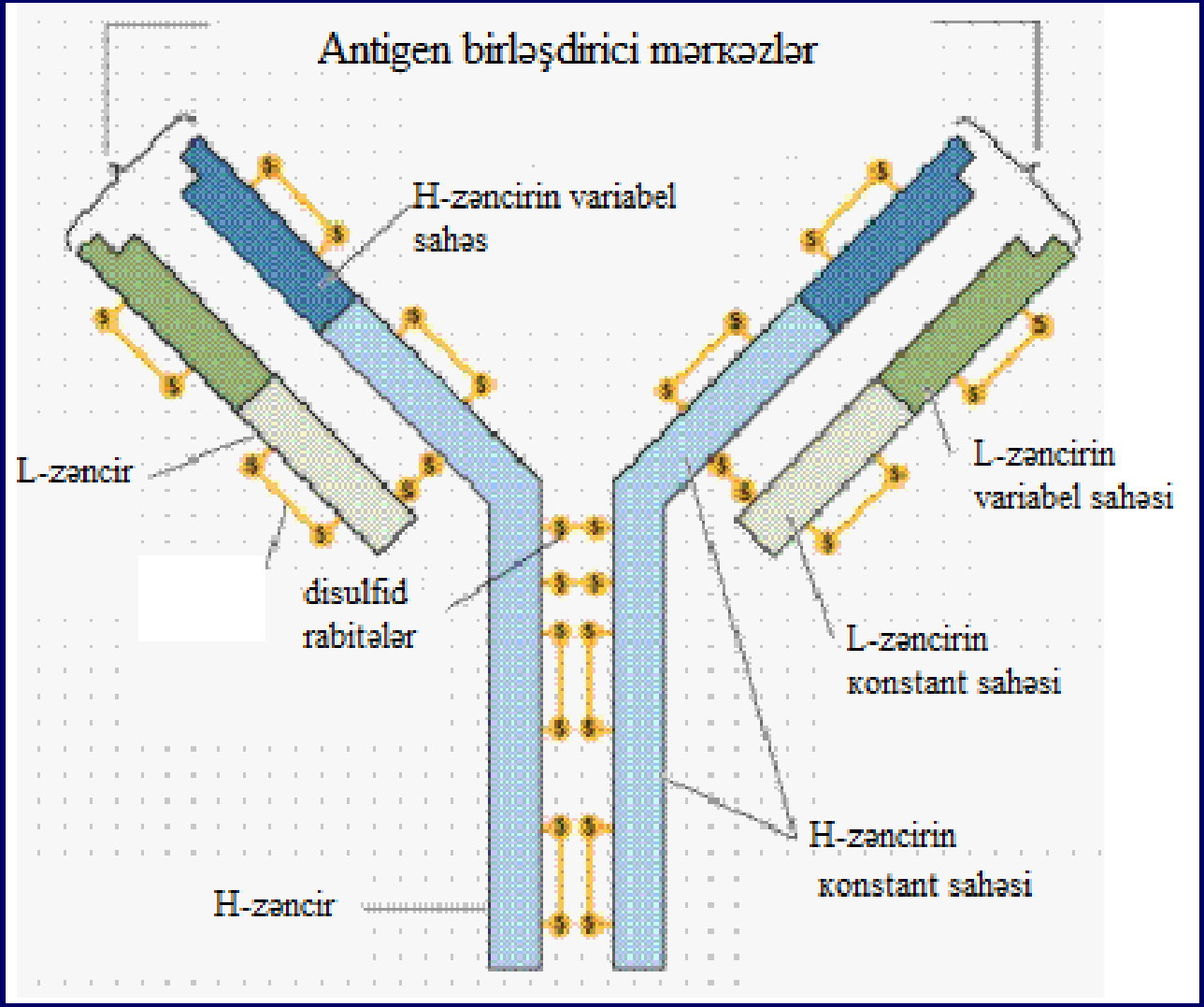
Immunoglobulins or antibodies

- Cooperation of three cells –macrophages, Th- and B- lymphocytes is essential for antibody synthesis.
- After processing antigens are expressed in cell surface in association with MHC II proteins
- Th- Lymphocytes produce - IL2 (T-cells growth factor), IL4 (B-lymphocytes growth factor) and IL5(B-lymphocytes differentiation factor). These cytokynes activate antigen specific B-lymphocytes. Activated B-lymphocytes proliferate and differentiate into plasma cells producing immunoglobulins(antibodies).

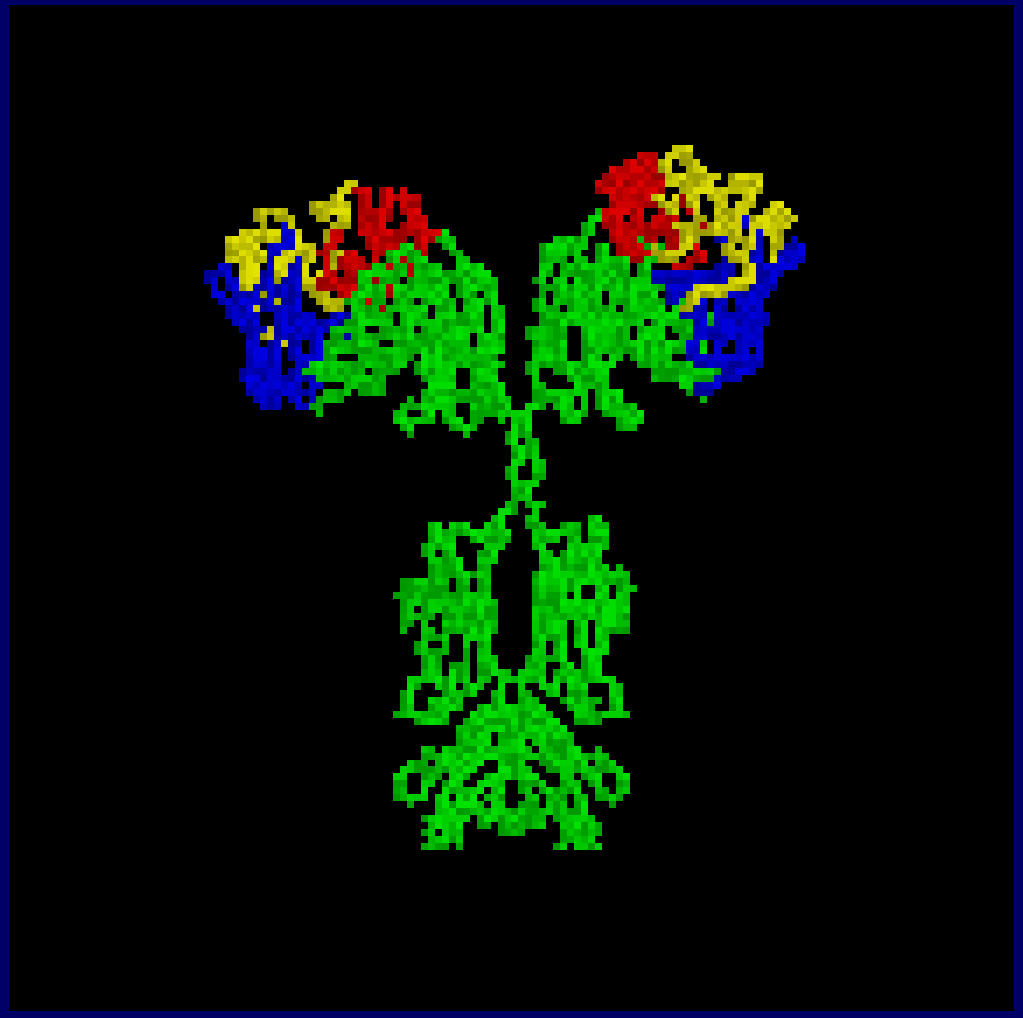
Immunoglobulins

- Immunoglobulines (Ig) are gamma-globulin fraction protein.
- The Ig monomer is composed of twolight
- (L) and two heavy (H) -4 polypeptide chains joined together by disulfide bonds.
- The molecular weight of light chains is 25,000, and heavy chains is 50,000- 70,000. L- and H-chains are divided into two regions called variable -variable (V) and constant -constant (C).

Immunoglobulin structure



Immunoglobulin model



Imunoglobulin structure

- L- and H-chain terminal regions have variable (***hypervariable***) aminoacids (VL, VH).
- Hypervariable region consists of 5-10 aminoacids and form antigen binding syte. This region is called *Fab*-**fragment** (*fragment antigen binding*) and responsible for binding with antigen.
- Ig-molecule binds to antigen with noncovalent electrostatic, van-der-vaals, hydrogen and hydrophobe bonds.

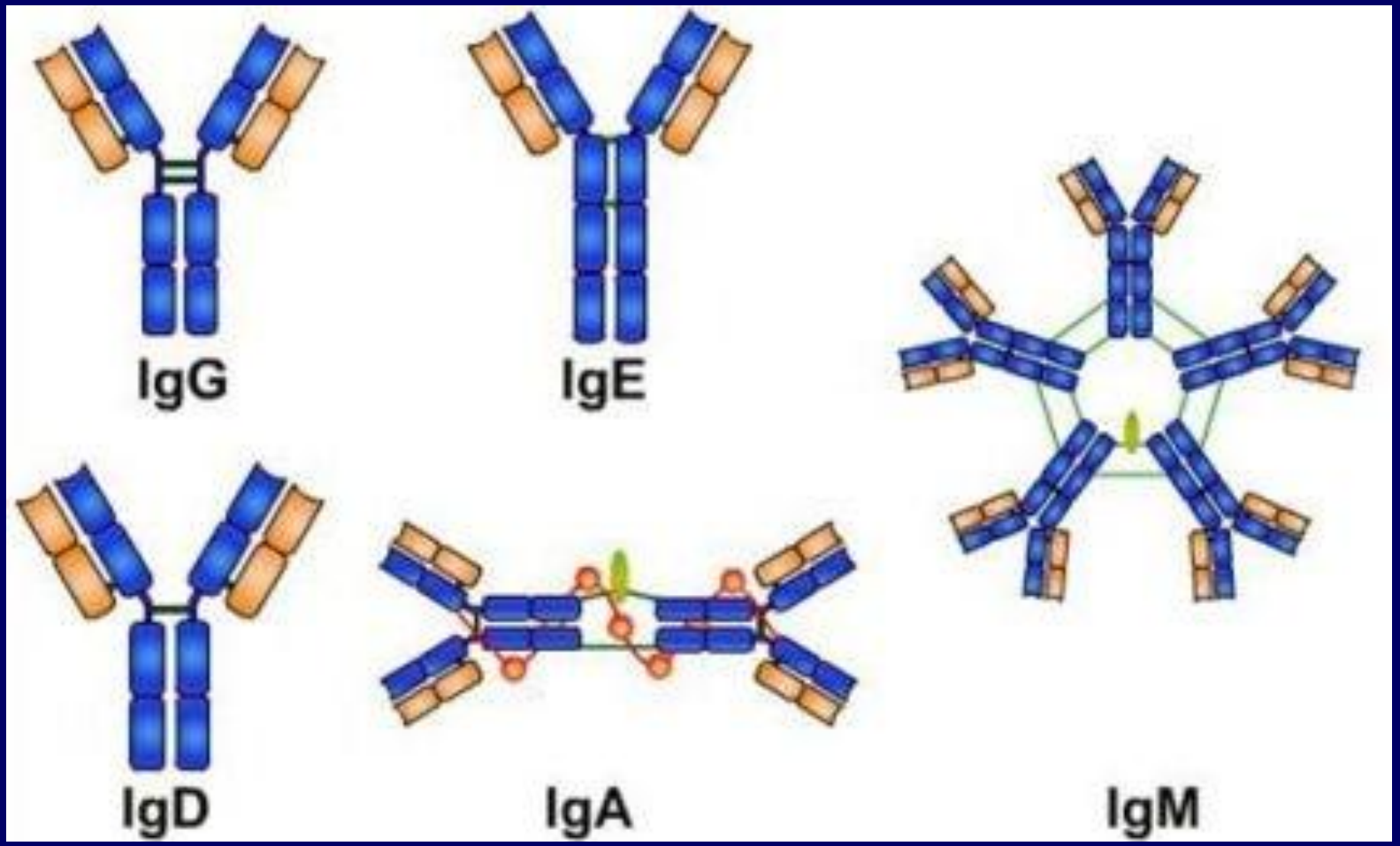
Immunoglobulin structure

- **H and L chains have constant domens called *Fc*- fragments (*fragment crystallisable*) with different function.**
- **This fragment is able to bind with complement and cells (macrophages, mast cells, lymphocytes).**
- **Antibody molecule is broken down by proteolytic enzymes(papain) to 2 fragments: 2 Fab and 1 Fc fragments.**

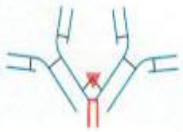
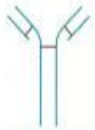
Immunoglobulin classes

- **Depending on antigenic features 5 classes of H- chain exist - α, μ, γ, ε, δ.**
- **Accordingly, 5 classes of immunoglobulins are distinguished. Antibody with α-type chain is called IgA, μ-chain- IgM, γ-chain -IgG, ε- IgE, δ-chain- IgD.**
- **Some classes of Ig have subclasses: IgG - 4 (IgG1, IgG2, IgG3, IgG4), IgA, IgM and IgD classes have 2 subclasses.**

Immunoglobulin classes



Immunoglobulin classes

Immuno-globulin Class	Structure	Molecular Weight	Percent in Blood	Location	Crosses Placenta?	Fixes Complement?
IgG		150,000	75–80	Blood and tissue fluids	Yes	Yes
IgM		900,000	6–7	Blood and tissue fluids	No	Yes
IgA		170,000*	15–21	Saliva, mucus, and secretions	No	No
IgE		200,000	<1	Skin, respiratory tract, and tissue fluids	No	No
IgD		180,000	<1	Serum	No	No

G immunoglobulin (IgG)

- Ig G have molecular weight of 150000 Da and consists of 2 Light (L) and 2 Heavy (H) chains connected to each other by disulfide bonds.
- Ig G makes up 70-80% of plasma immune globulins.
- Synthesized by B-lymphocytes and plasmatic cells.
- It is detected during primary and secondary immune response.

G immunoglobulin (IgG)

- IgG antibodies are dominant during secondary reactions and have importance in bacterial and viral infections.
- IgG is the only antibody that can pass the placenta: its Fc fragment is able to bind to receptors on the surface of placental cells. Thus , the concentration of IgG in the serum of newborns is higher than that of other immunoglobulins.
- IgG is one of two immunoglobulins that can activate the complement (the second is IgM). The half-life of IgG is 21 days. IgG is an opsonizing immunoglobulin.
- Like IgE antibodies IgG has cytophilia (tropism against mast cells and basophils) and is involved in the development of type I allergic reactions..

M immunoglobulin (IgM)

- It is the largest among all immunoglobulin molecules. Its pentamer structure – ie 10 antigen-binding centers, enales it to bind 10 antigens.
- The molecular weight is close to 900,000 D. Has subtypes M1 and M2.The heavy chain of the IgM molecule, unlike other isotypes, has 5 domains.
- The half-life of IgM is 5 days.
- It accounts for 5-10% of all serum immunoglobulins. The average level of IgM in the blood serum of a healthy adult is about 1 g/l.

M immunoglobulin (IgM)

- Ig M is synthesized by B-lymphocites and progenitors. Phylogenetically Ig M is the oldest immunoglobulin class.
- It is produced at the beginning of primary immune response and in organism of newborns.
- It is detected in organism of newborn from the 20th week of intrauterine development.
- It does not pass the placenta. Detection of immunoglobulins of the isotype M in the blood serum of newborns indicates intrauterine infection or placental abruption.

A immunoglobulin (IgA)

- It makes up 10-15% of immunoglobulins of blood serum. Has two subclasses - IgA1 and IgA2.
- IgA1 is present in the serum, while IgA2 is a part of sIgA and predominant in the secretions. IgA2 is resistant to the action of proteolytic enzymes of saliva, secretions of intestinal mucosa.
- The secretory component of sIgA protects the immunoglobulin molecule from the action of proteolytic enzymes of secretions.

Secretory IgA (sIgA)

- Secretions of mucous membranes have sIgA with two monomeric immunoglobulin A (IgA) molecules (molecules) connected to each other by a J-chain (English, join - binding) and an S-chain (secretory component).
- Each secretary IgA molecule (molecular weight - 400,000) consists of two H, two L-chains and one molecule J-chain. It is present in IgA and IgM-molecules as well, these immunoglobulins are multimer (dimer and pentamer).

E immunoglobulin (IgE)

- It differs from other immunoglobulins by its high cytophilicity - ability to bind to mast cells and basophils.
- This immunoglobulin has two important biological features:1) it provides immediate type hypersensitivity reactions;2) it is involved in the body's defense response during parasitic diseases, especially helminthiasis (worm infestations),

E immunoglobulin (IgE)

- Fc-fragment of IgE binds to surface of mast cells and basophiles. Antigens (allergens) bind to this comple causing release of mediators from these cells and development of immediate type hypersensitivity.
- Although IgE amount in the serum of healthy people is very low (about 0.002%), in allergic conditions its amount increases significantly, and can even be detected in the secretions. IgE does not have the ability to bind to the complement and does not pass the placenta.

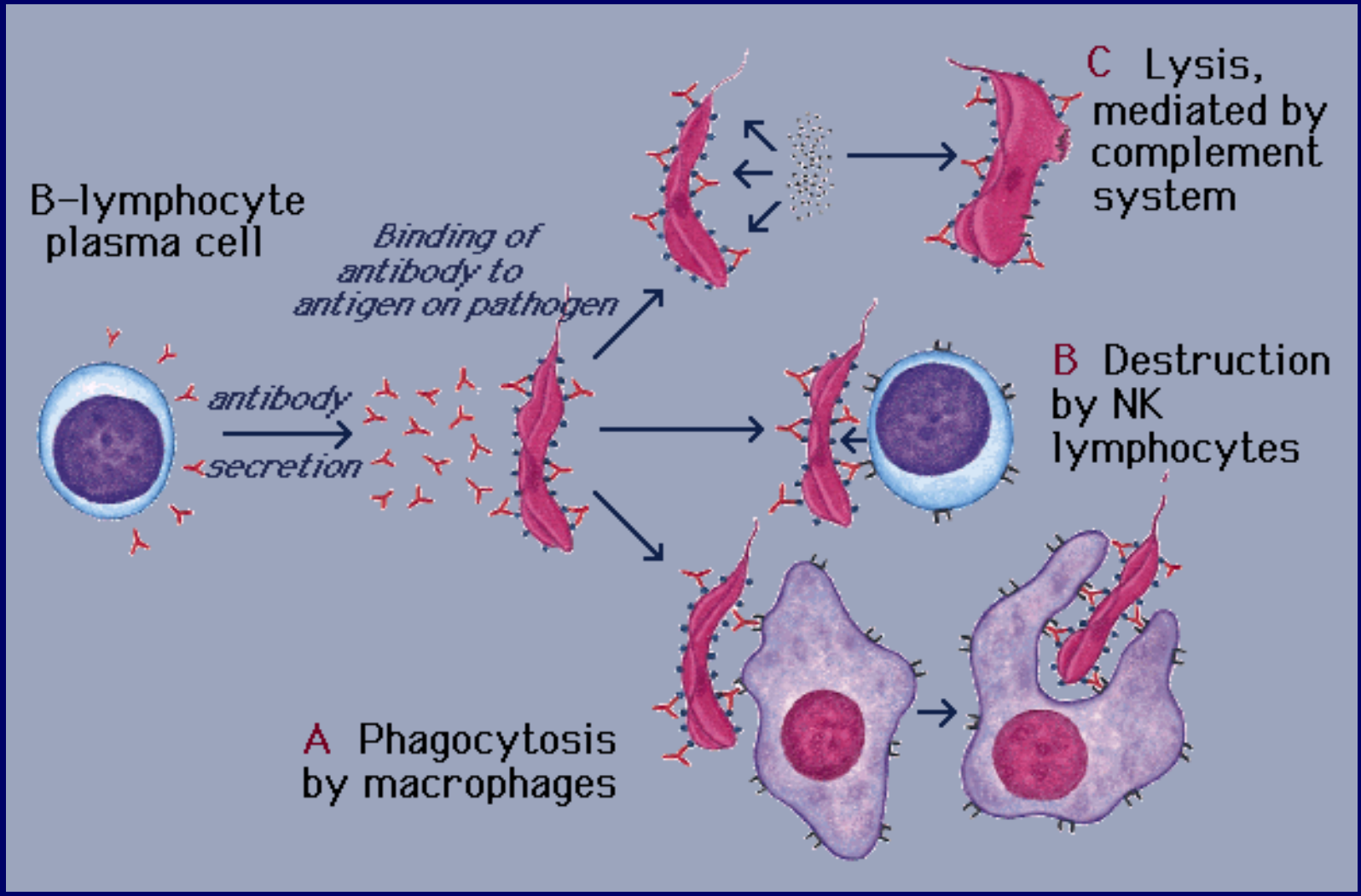
E immunoglobulin(IgE)

- IgE is important in the protection of the organism against a number of helminthiases and other parasitic diseases.
- Due to their large size, helminths and parasites can not be ingested by phagocytic cells. They are destroyed by special enzymes produced by eosinophils. Specific IgE produced in response to helminth antigens binds to eosinophil receptors, thus forming an immune response accompanied by antibody-dependent cellular cytotoxicity.

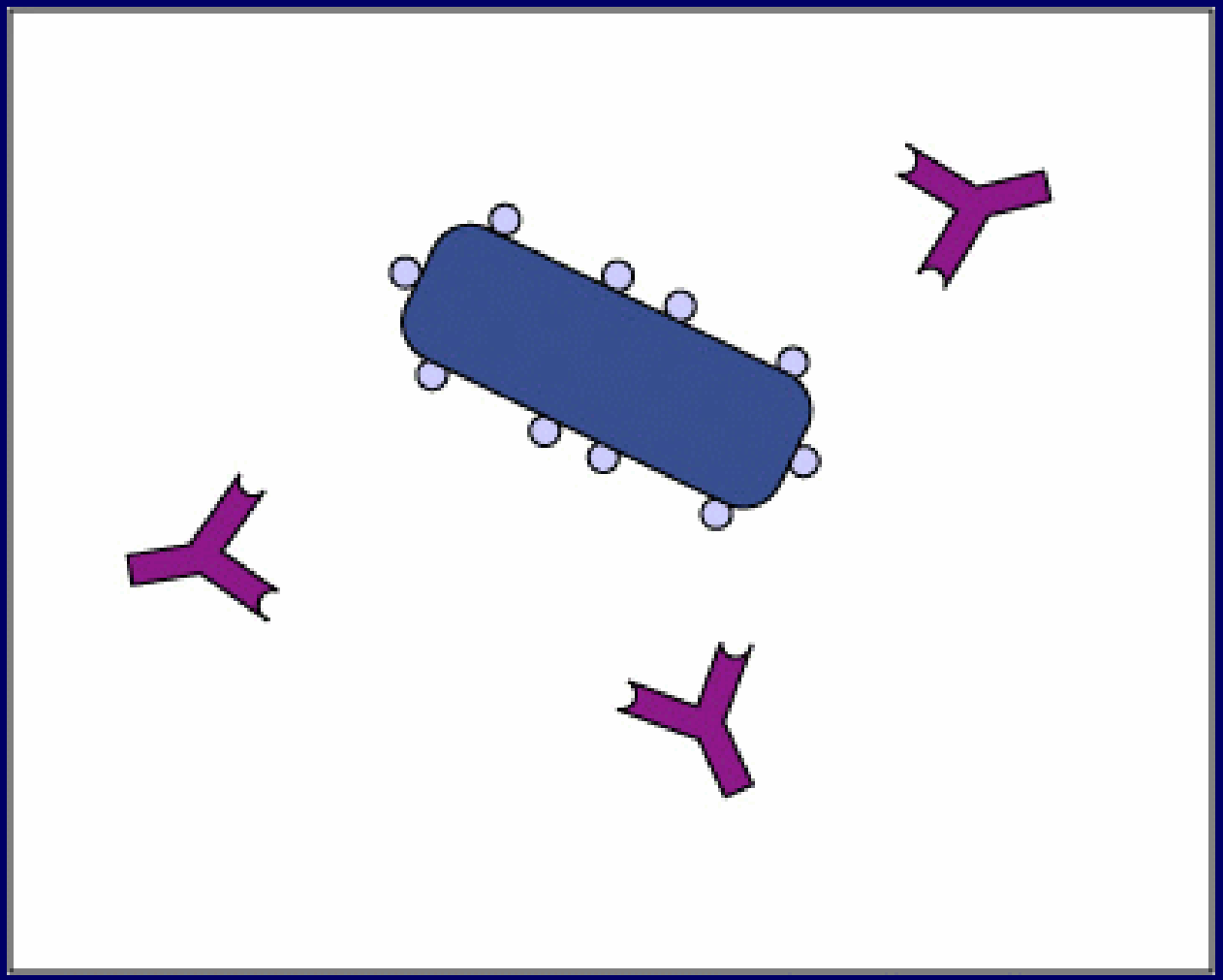
D immunoglobulin (IgD)

- The antibody function of this immunoglobulin is unknown, but it acts as an antigen-receptor on the surface of the precursors of B-lymphocytes.
- IgD is present in blood serum in small amounts - 0.03 g / l (0.2% of all circulating immunoglobulins).
- It has a molecular weight of 160,000 D and is a monomer.

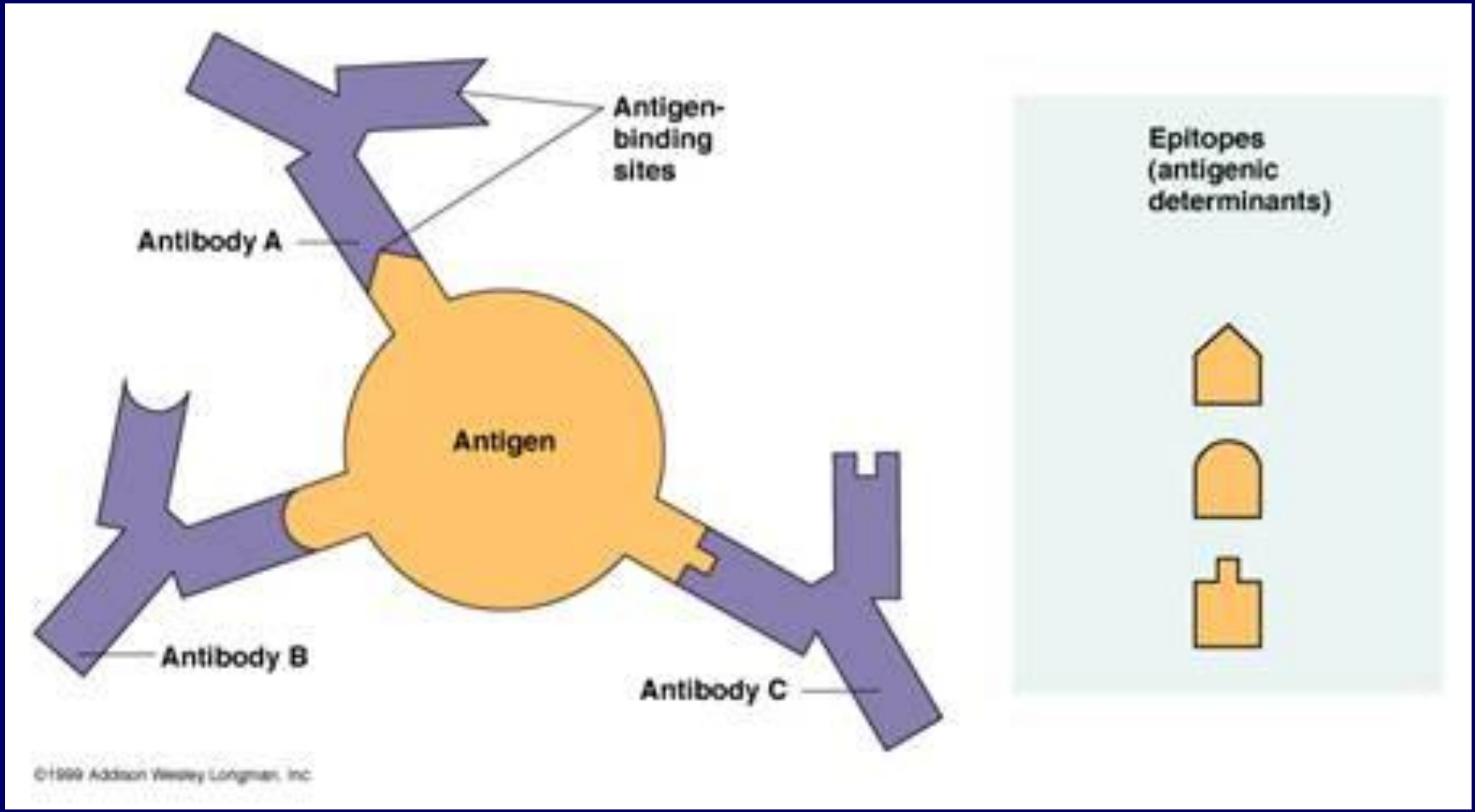
Antibodies function



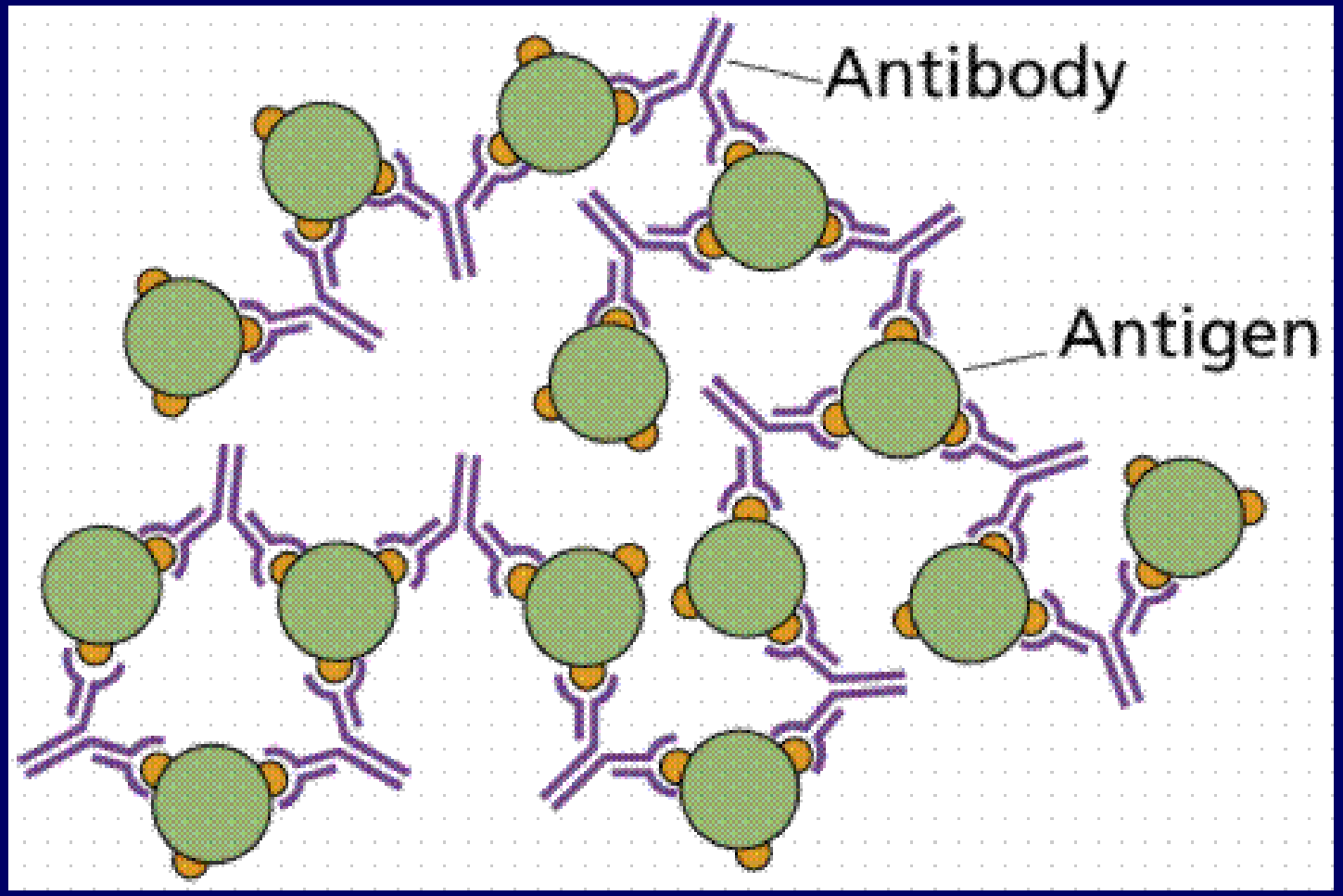
Opsonization function of antibodies



Specificity of antibodies



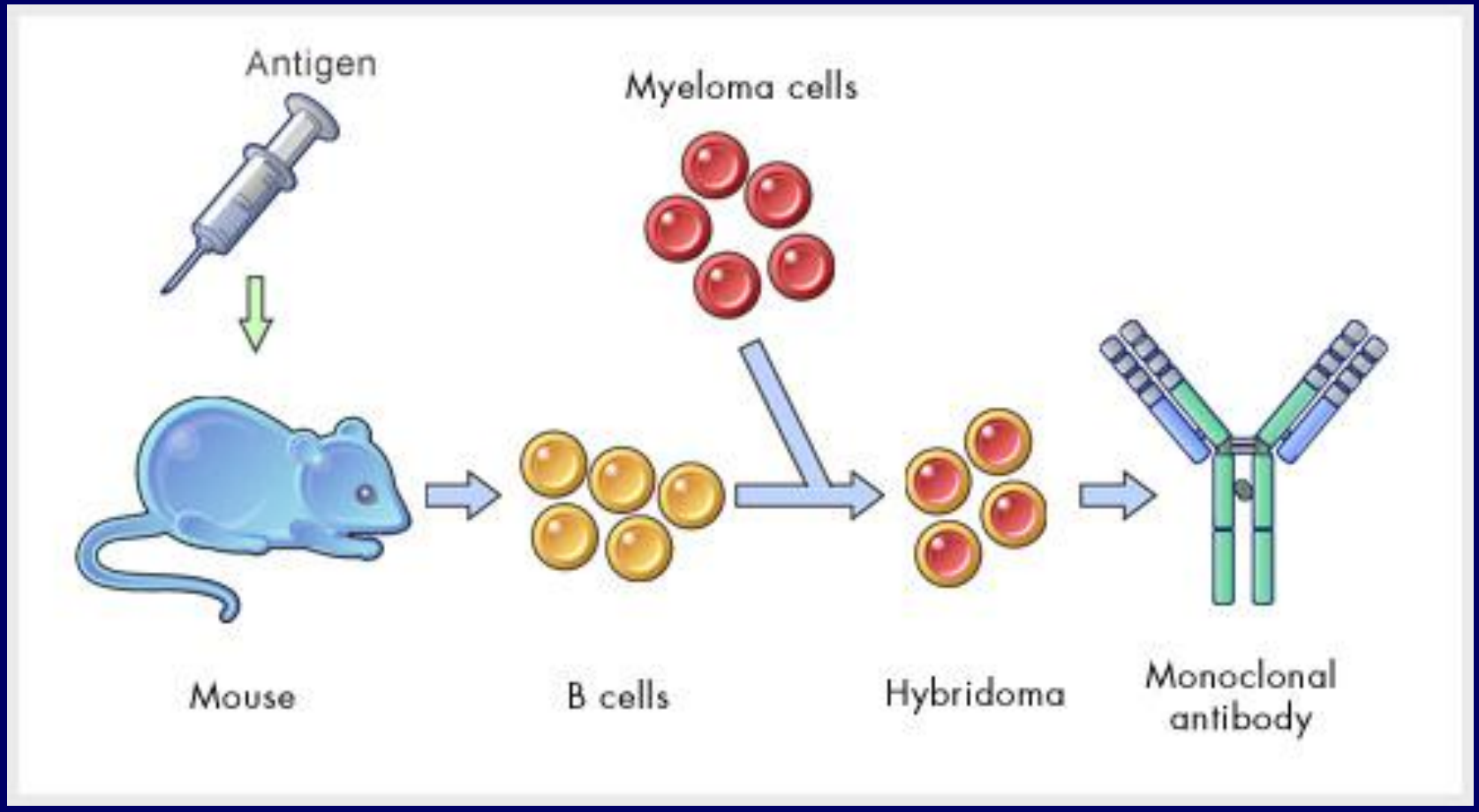
Agglutination phenomenon



Diversity of antibodies

- **Normal, or natural antibodies**
- **Receptor immunoglobulins**
- **Policlonal antibodies**
- **Monoclonal antibodies- D.Keller andT.Milstein in 1975 obtained hybridoma by synthesis of antibody and attaching it to B- lymphocyte myeloma cells.**
- **Noncomplete, blocking antibodies**

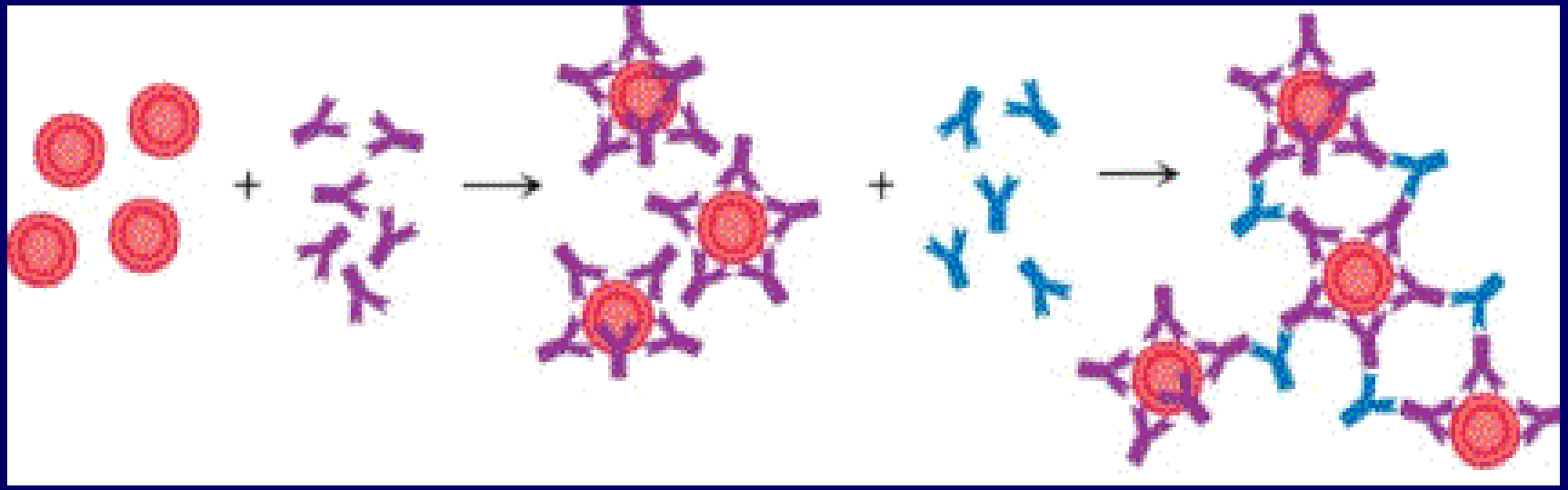
Obtaining monoclonal antibodies



Incomplete or Blocking antibodies

- **Sometimes, due to the absence of one of the active centers in the Ig molecule, they combine with the antigen only with one center. Thus, there is no formation of large aggregates.**
- **Therefore, such antibodies are called incomplete or blocking antibodies.**
- **Incomplete antibodies are detected by *Coombs reaction*.**

İncomplete antibodies are detected by Coombs reaction – using antiimmunoglobulin antibodies



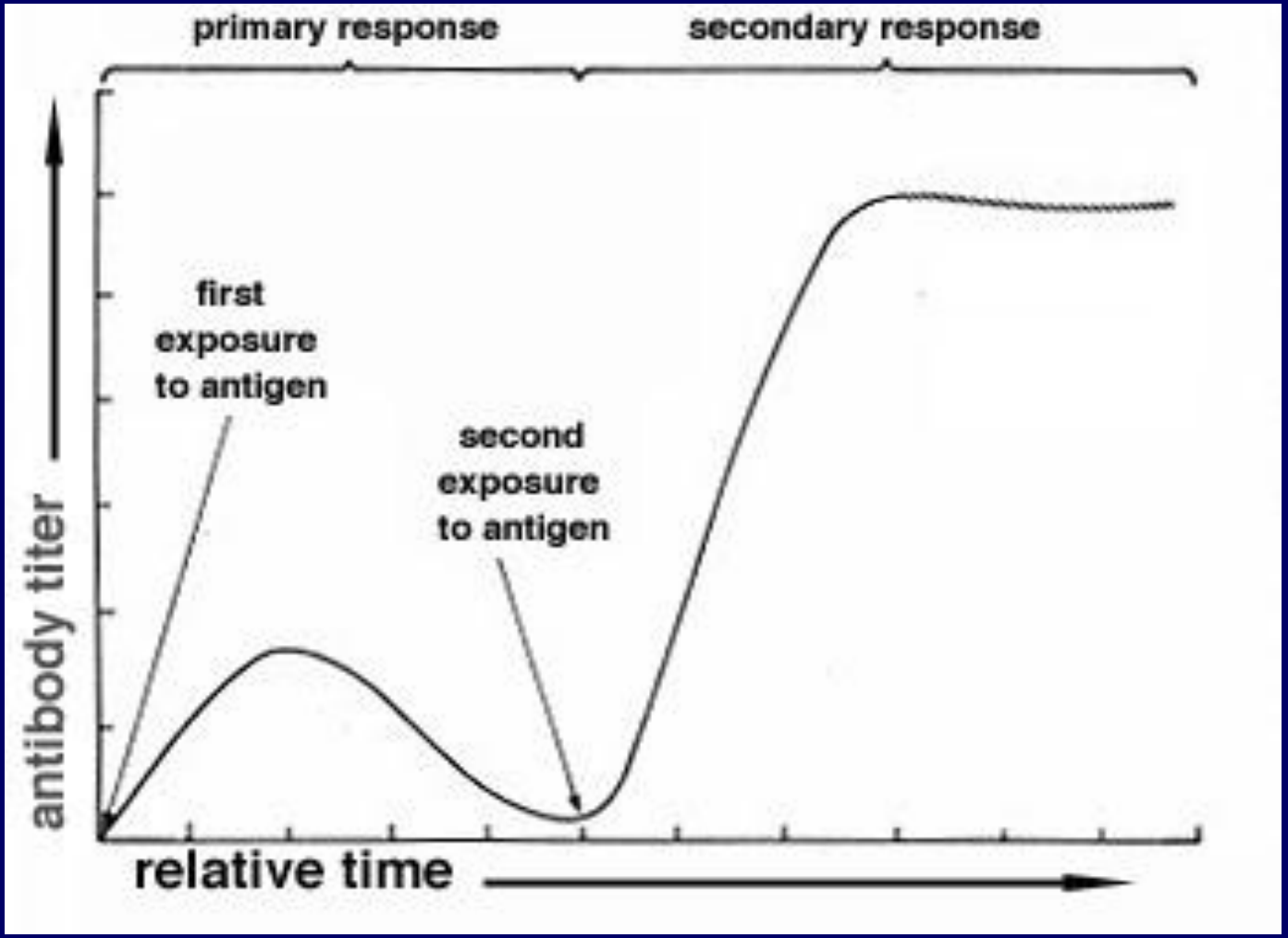
Affinity and avidity

- ***Affinity*** **refers to the strength with which the epitope binds to an individual paratope (antigen-binding site) on the antibody.**
- ***Avidity*** **refers to the measure of the total binding strength of an antibody at every binding site. Among the different immunoglobulins that have the same affinity, antibodies of class M are more avid, as it has 10 antigen-binding centers.**

Antibody (specific immunoglobulins) production dynamics

- *Primary immune response.* ***Antibodies can be detected in plasma 4-5 days (sometimes 7-10 days)after antigen exposure.***
- *Secondary immune response*. **Exposure to same or similar(cross reactive) antigen after several months results in formation of faster and stronger immune response (in comparison to primary).**

Antibody production dynamics



Obtaining of hyperimmune serum

- The phenomenon of intensive production of antibodies during secondary immune response is used in medicine to create and maintain immunity.
- First formation of immunological memory is induced by vaccine. Then, revaccination is performed in order to maintain immunity. This phenomenon is also used to obtain hyperimmune serums containing high titer of antibodies. For this purpose, human and animals are immunized (hyperimmunization) by antigens using special schemes.

Theories about the diversity of antibodies

- **P.Erlich proposed i*«side chains»* theory (1898)**
- **F.Bernett *clonal-selection theory* (1959)**
- **H.Jerne *«immune network» theory***
- **S.Tonegawa *molecular-genetic theory* (1983)**

İmmune response reactions, their types and mechanisms

Types of immune response

- Antibody production,
- Immune phagocytosis
- Antibody dependant and nondependant cytotoxicity
- Hipersensitivity reactions,
- Immunological memory,
- Immunological tolerance.

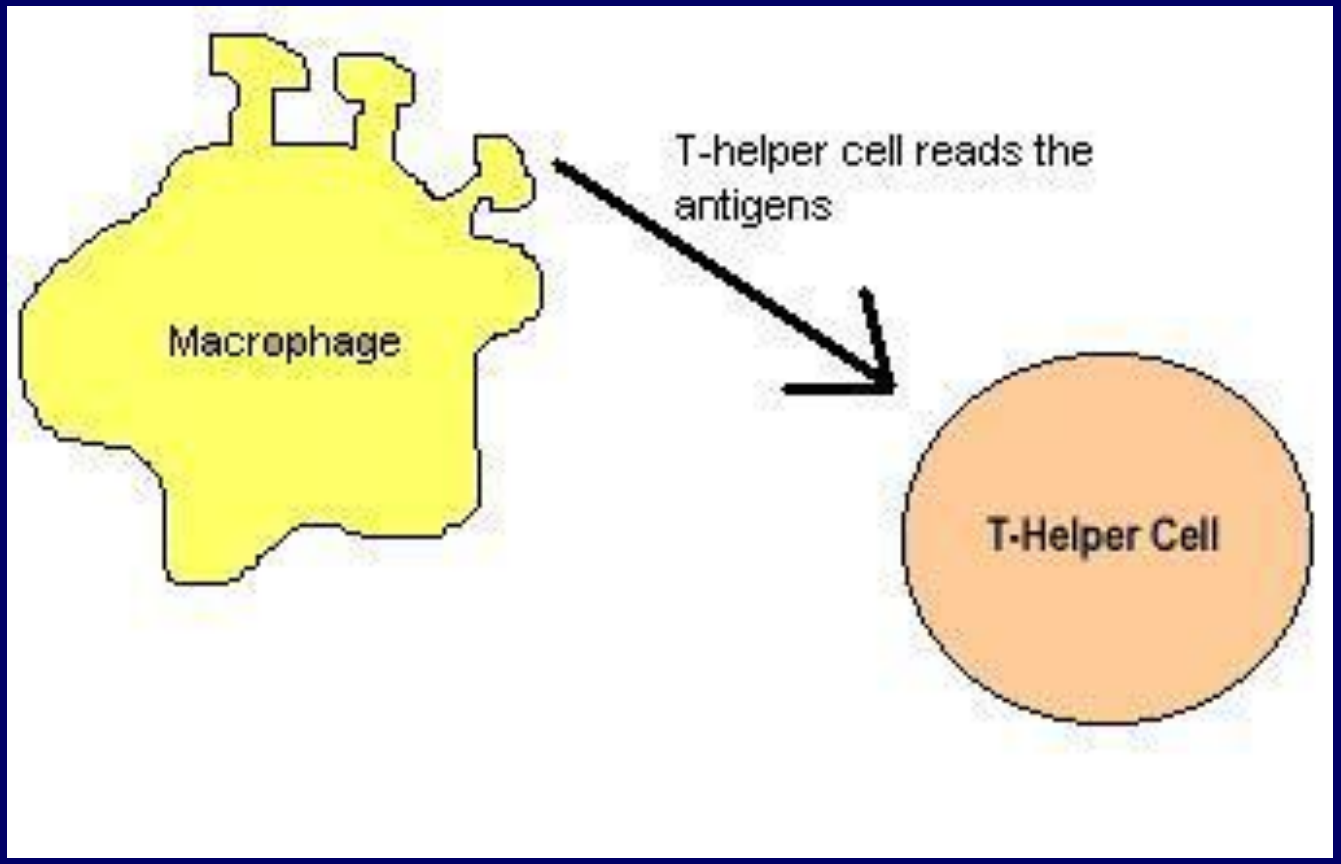
Immune response mechanism

- **The first stage of the immune response is the process of antigen presentation by macrophages. The presentation is complex of reactions including phagocytosis of antigen, its "processing" in the phagolysosomes of these cells, and the presentation of the antigen to T0-helpers in combination with class II MHC.**
- **As a result, T0-helpers differentiate into Th1 and Th2 cells, in other words, cause the development of different types of immune responses.**

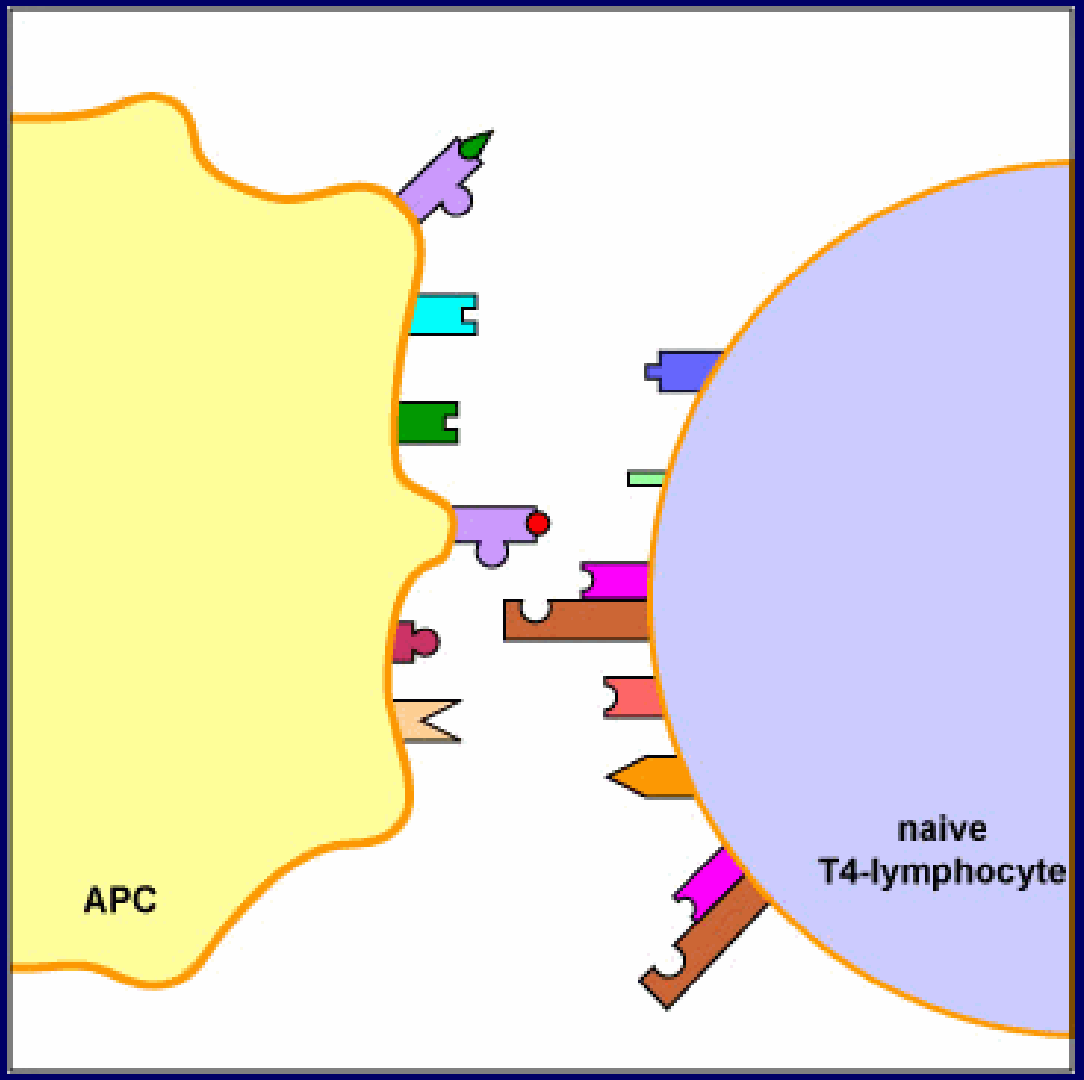
Immune response mechanism

- ***T1-helpers are responsible for*** **cellular immune response, delayed type gypersensitivity, production of IL-2, -3, gamma-IFN, timor necrosis factors (TNF) etc.**
- ***T2-helpers produce interleukins of*** **humoral immunity, and immediate type of hypersensitivity - IL-4, 5, 6, 9, 10, 13 etc.**

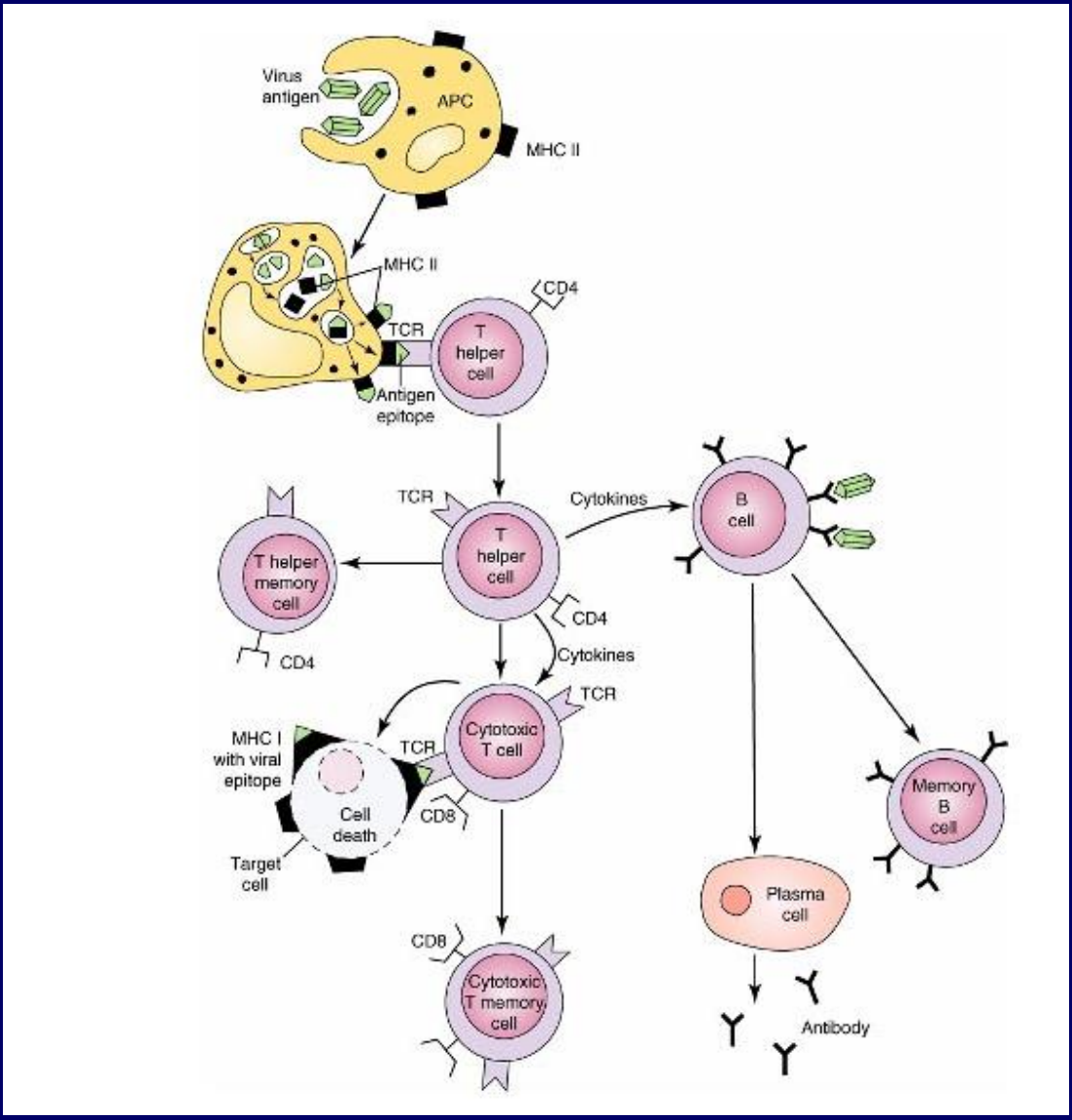
Immune response mechanism

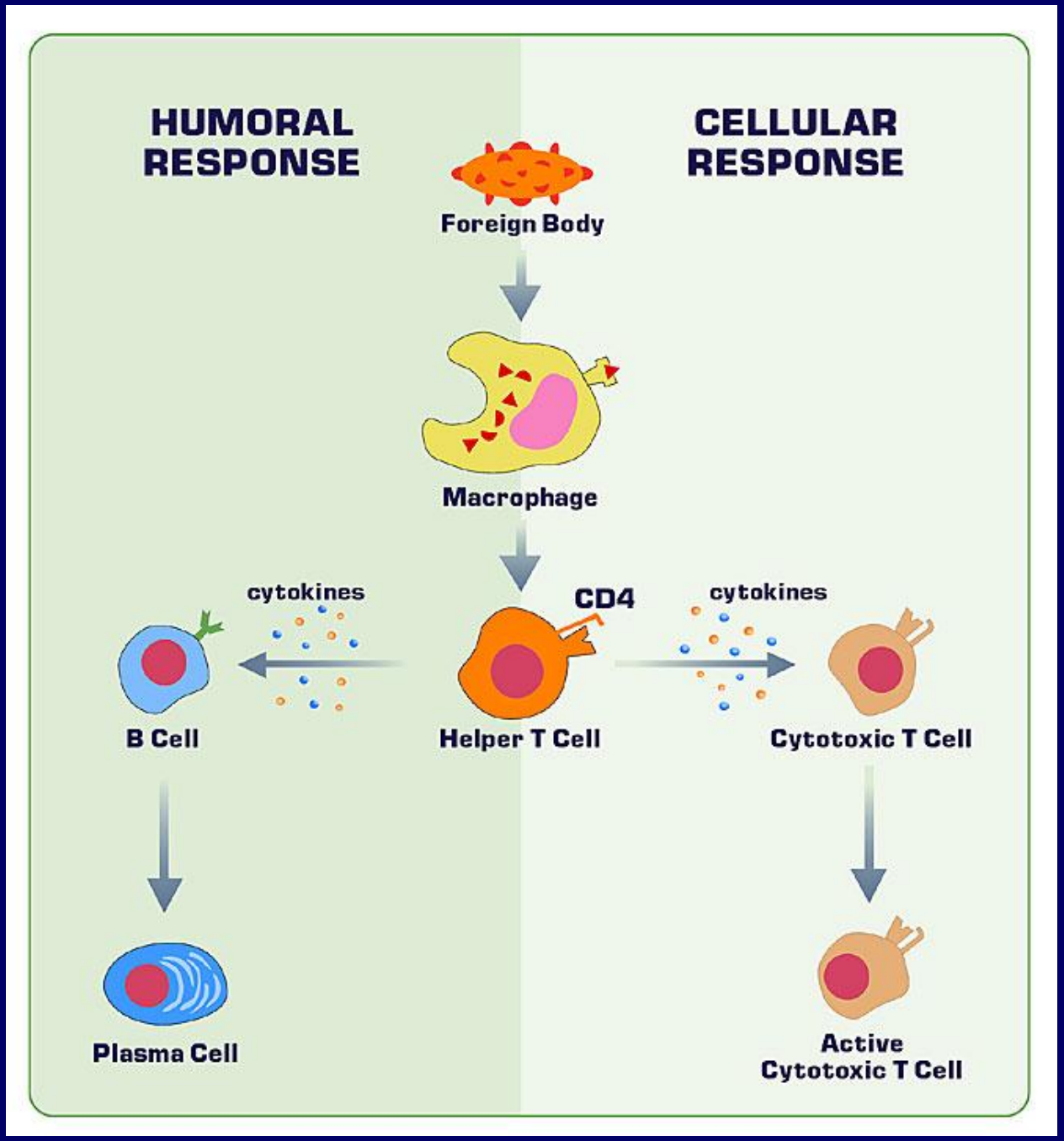


Immune response mechanism



Immune response mechanism

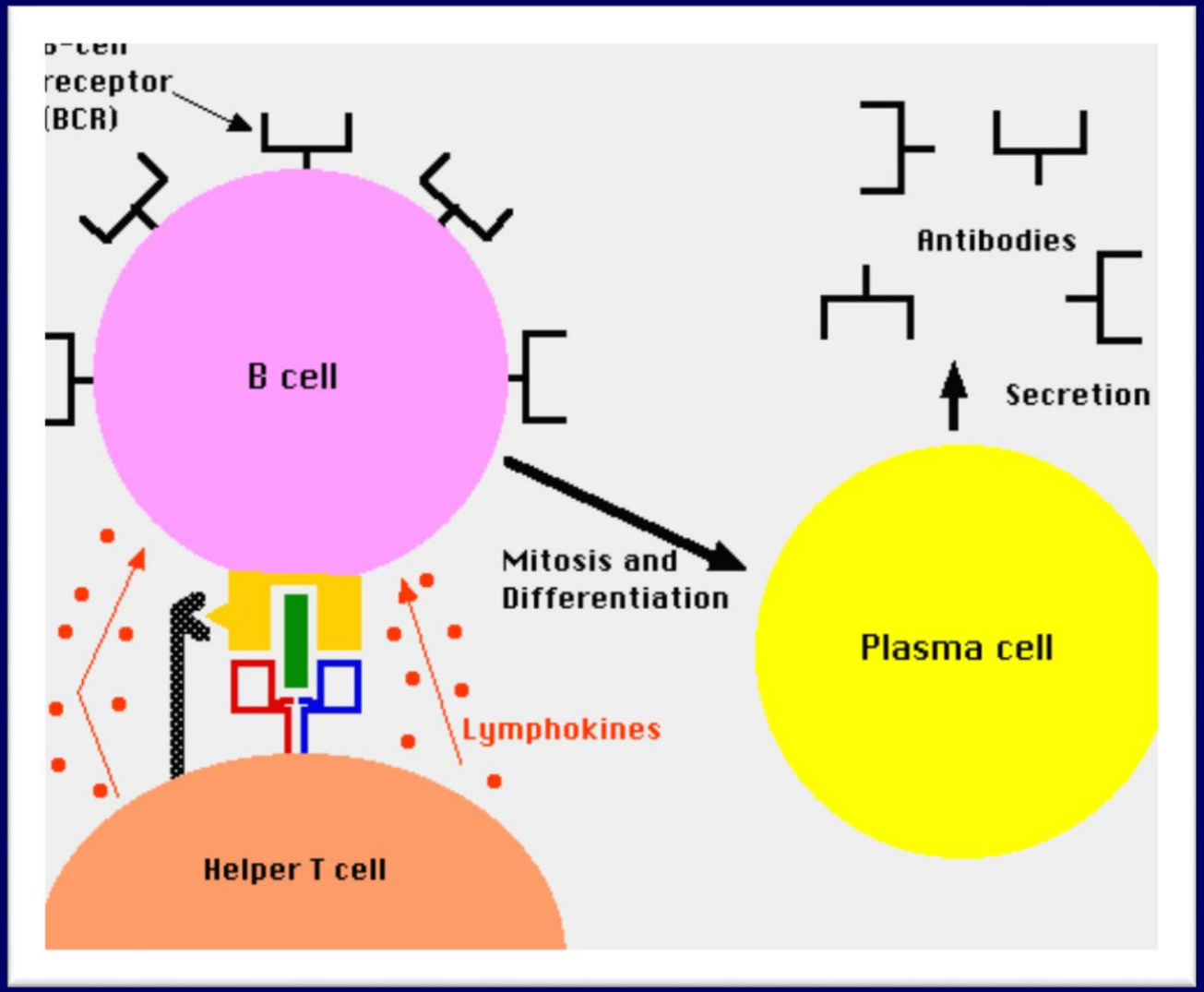



HUMORAL RESPONSE
CELLULAR RESPONSE
Foreign Body
Macrophage
cytokines
CD4
cytokines
B Cell
Helper T Cell
Cytotoxic T Cell
Plasma Cell
Active Cytotoxic T Cell

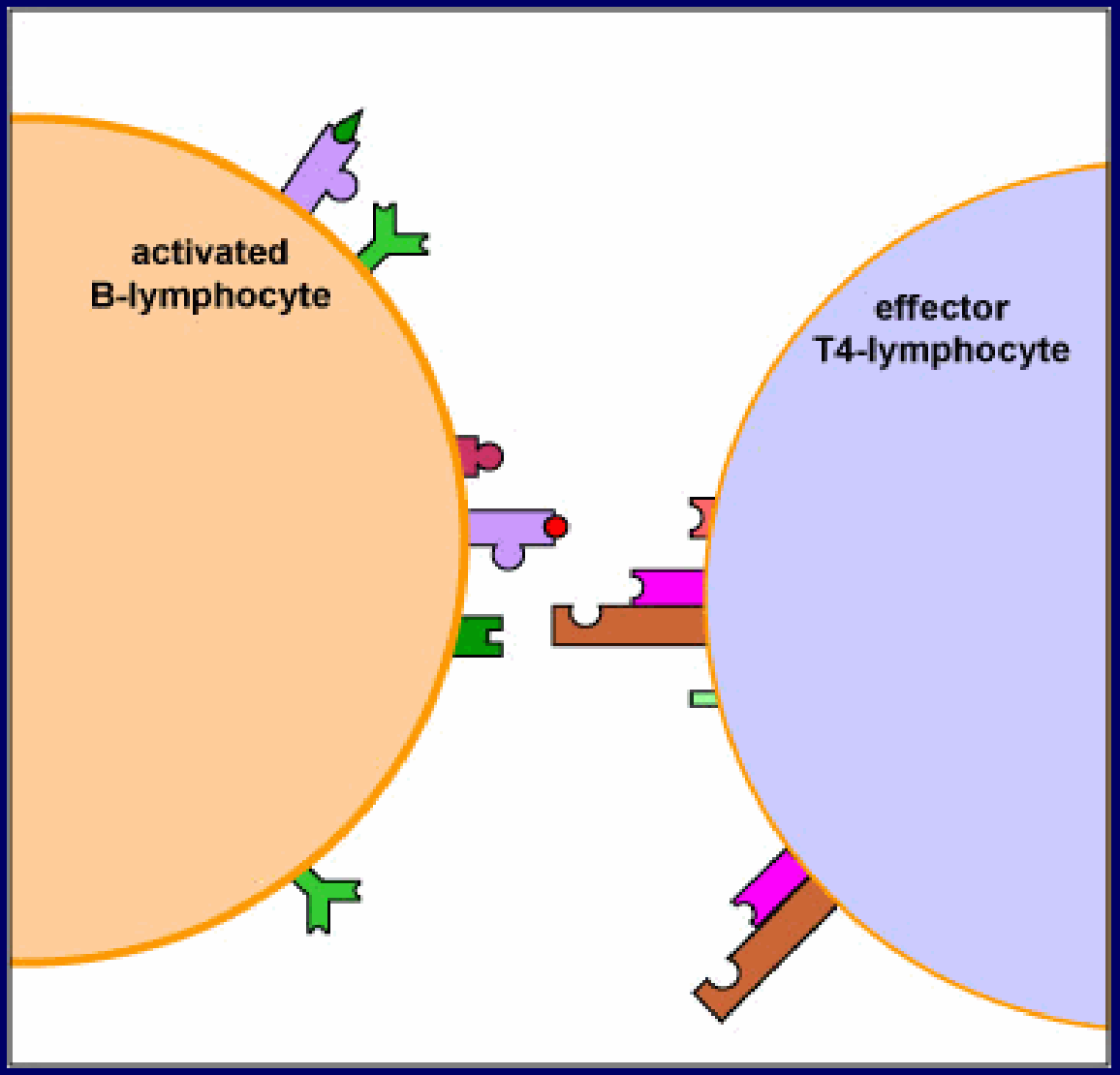
Humoral immunity

- Production of antibodies by B-limphocytes and plasma cells.
- Neutralization of antigen by antibodies and complement system

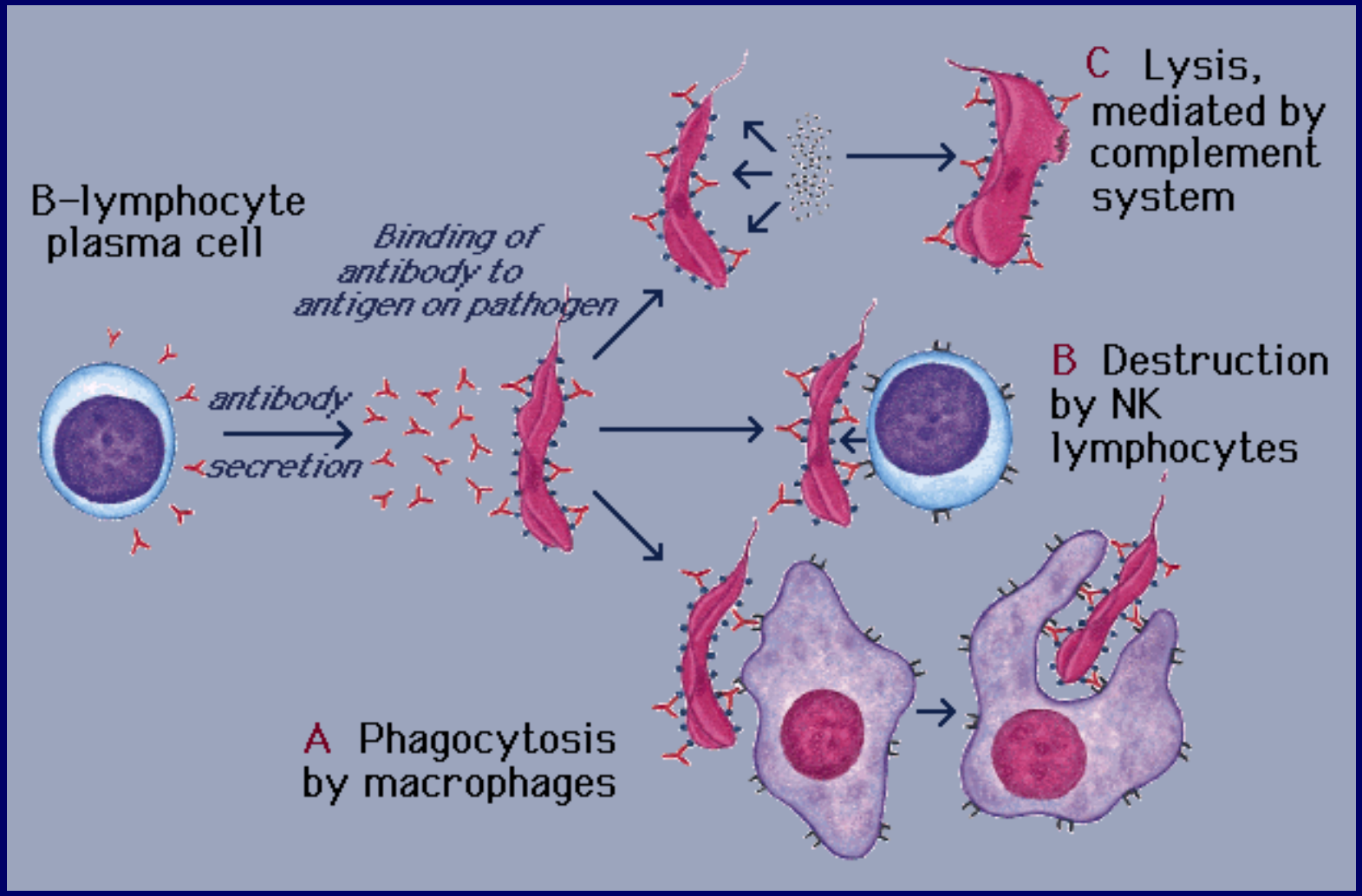
Mechanism of humoral immunity



Mechanism of humoral immunity



Mechanism of humoral immunity



Mechanism of cellular immunity

- ***T-killers possess on surface receptors against antigens*.**
- ***T-killers recognize antigens in complex with* MHC I molecule. By doing so, "native" cells are distinguished from "foreign" cells.**
- **T-killer destroys target cells with antibody-independent cell cytotoxicity (AiCC).**
- **In this case, the target cells there is no need for binding of antigens with the corresponding antibodies. This process is carried out by a number of toxic substances - perforin, granzym and granulisin.**

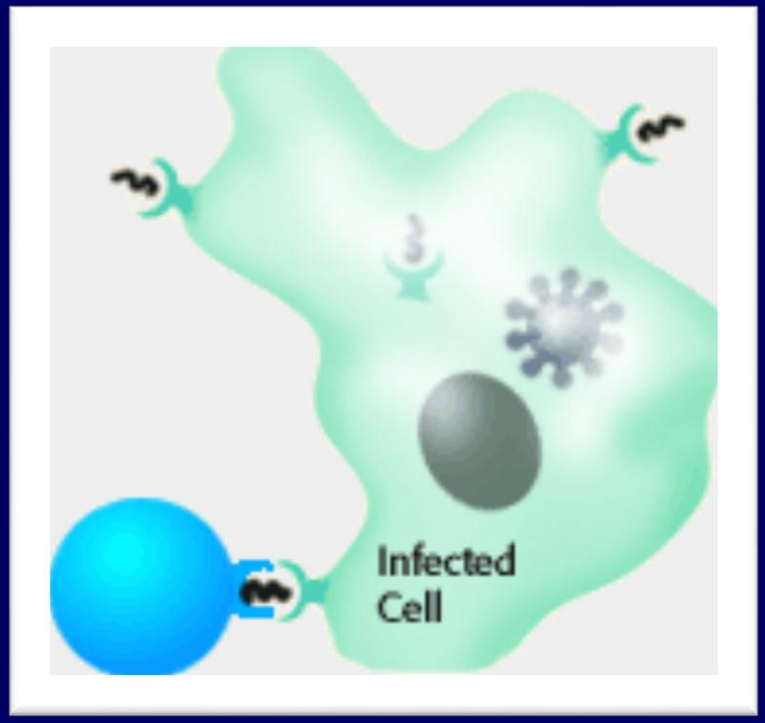
Mechanism of cellular immunity

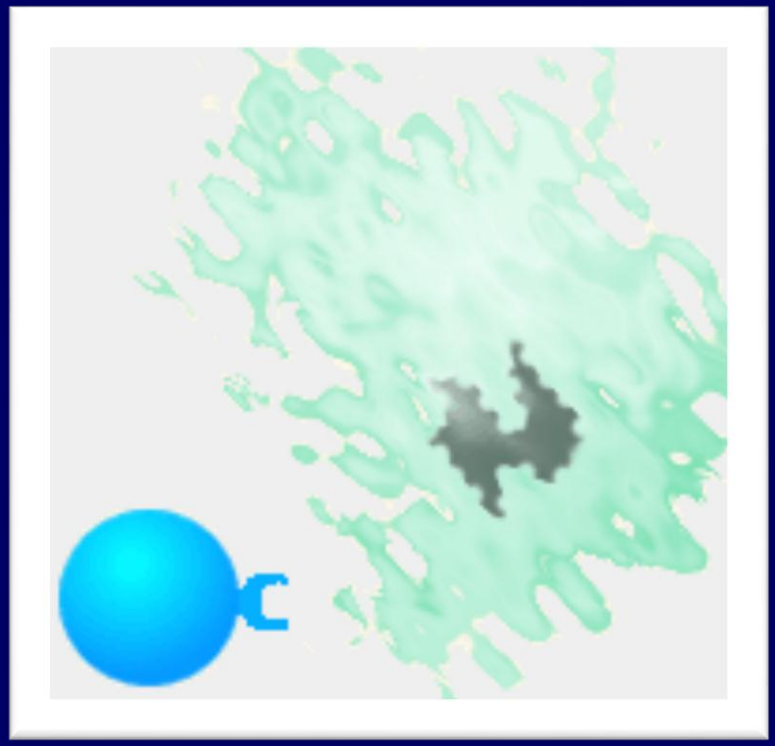
- **Perforin is a toxic protein that is synthesized by T-killers other cytotoxic lymphocytes, including natural killers. This substance is located In the cytoplasm of T-killers in the form of granules in the vicinity of the TCR.**
- **After binding of TCR to target cells the content of granules is release the cleft between the cytotoxic lymphocyte and the target cell.**
- **Perforin penetrates the cytoplasmic membrane of the target cell. Here it polymerizes to form transmembrane pores. This causes lysis of the target cell, as well as the transfer of other toxic substances to the target cell – granzymes and granulysins.**

Mechanism of cellular immunity

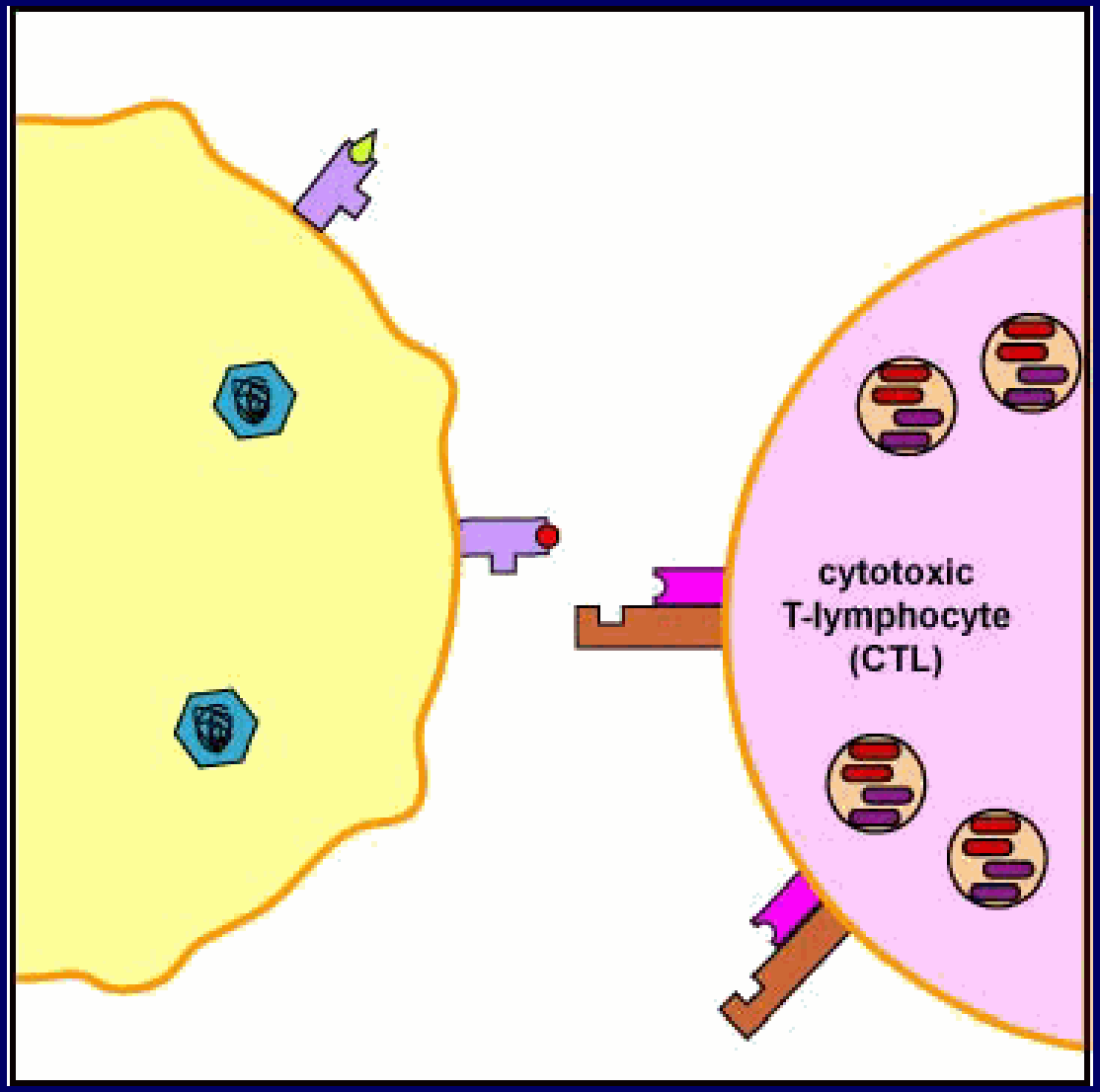
- **Granzymes and granulysins are synthesized and then accumulate in the granules.**
- **They enter the target cells from the pores formed by the perforin, destroying them by inducing the apoptosis of the target cells.**

Destroing of target cells by cytotoxic T-lymphocites





Destroing of target cells by cytotoxic T-lymphocites



İmmunological memory

- After primary immune response an immunological memory is always formed.
- immunological memory supports effective immune reaction during repeated exposure of the immune system to the same antigen.
- Implementation of Vaccination in medical practice is based on these features of the immune response.
- When vaccinated people are infected by pathogenic microorganisms, the immune response develops rapidly and intensively preventing development of disease.

“Memory” cells

- Immunological memory is provided by "memory cells".These cells can be from both T- and B-lymphocyte populations.
- Memory cells do not differ from other lymphocytes in morphological features, but they have long-lasting changes at genetic level.
- Memory cells have specificity against the antigens they come into contact first time.
- These cells are formed against common antigens, especially after frequent exposures. As the number of exposures of the immune system to antigens increases, memory cells accumulate in the organism.

İmmunological tolerance

- **Tolerance – is a state of specific unresponsiveness of immune system, i.e. immune system does not form immune response against specific antigen (or epitope).**
- **Commonly exposure to antigens in embryonic periodic does not induce immune response. Exogen substance injected in embryonic period are accepted as «self» tissue.**
- **In thymus autoreactive T-cells progenitors attacking self cells are destroyed.**
- **Tolerance formed inside the thymus is called central tolerance while tolerance formed outside the thymus – periferic tolerance.**

İmmunological tolerance

- The tolerance of B-cells against self antigens can develop by two mechanisms:
- 1) clonal deletion - most likely as a result of removal of autoreactive precursors of B-cells in the bone marrow;
- 2) B-cell anergy in peripheral organs of the immune system.

Factors supporting immunological tolerance

- **Immaturity of the organism- for example, newborn animals are not immunologically mature and do not respond adequately to foreign antigens;**
- **Structure and dose of antigen**
- **- Simple molecular antigens - tolerogens more easily induce tolerance.**
- **- Large doses of antigen also induce tolerance instead of the immune response.**
- **- When pure polysaccharides and amino acid polymers are injected in very large doses, "immunological paralysis" develops when there is no immune response.**

İmmunological tolerance

- T-lymphocytes have a more long lasting tolerance than B-lymphocytes;
- Cross-reacting antigens lead to limited tolerance;
- Prescribing immunosuppressive drugs enhances tolerance (for example, in transplant recipients);
- Tolerance lasts longer when there is an antigen that induces tolerance in the body.

Immunological tolerance and autoimmune diseases

- In adults there is a tolerance to tissue antigens exposured during embryonic period and recognized as “self” antigens.
- At some cases this tolerance is “lost” and ***autoimmune disease*** develops accompanied by immune response to self-antigens.