AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 18.

Acquired (specific) immunity. Antigens and their types. Antigenic structure of microorganisms. Antigens of the human body. Human immune system, organs and tissues, immunocompetent cells. Types of immune responses. Antibodies. Serological reactions, their application in microbiological diagnostics

FACULTY: General Medicine
SUBJECT: Medical microbiology - 1

Discussed questions:

1. Specific immunity and its kinds
2. Antigens, properties: foreignness (hetero-, allo-, iso-, autoantigens), antigenicity (epitopes), immunogenicity (complete and incomplete antigens), specificity (affinity).
3. Immunogens (T-dependent and B-dependent antigens, superantigens), allergens, tolerogens.
4. Bacterial antigens: somatic (O-), capsule (K-, Vi-) and flagella (H-) antigens, toxins, enzymes. Protective antigens.
5. Virus antigens: core and surface antigens.
6. The human antigens: blood group antigens, tissue compatibility antigens, CD-antigens.
7. The human immune system, its central and peripheral organs.
8. Immunocompetent cells (T- and B-lymphocytes, their subpopulations).
9. The mechanism of the immune response.
10. Forms of immune response: synthesis of antibodies, immune phagocytosis, cell killing, immunological memory, immunological tolerance, hypersensitivity reactions.
11. Antibodies, types (complete and incomplete antibodies), nature, structure, classes, types and functions.
12. Immundiagnostics, serological reactions and their application.

Purpose of the lesson:

To acquaint students with the body's immune system, immunocompetent cells, specific immunity, its cellular and humoral factors. Provide information about antigens and antibodies. Explain serological reactions and their role in microbiological diagnosis.

Specific immunity

- Depends on type of antigen entering organism with help of special factors.
- Defense factor created for any antigen cannot act on other antigens. Thus, this defense factor is specific

Antigens

- Genetically foreign substances stimulating specific immune responses (synthesis of antibodies, specific cellular immune response) are called antigens.
- Antigens may be both chemically pure (plasma albumin, egg albumin, purified microbial toxin) as well as complex drugs, cells and tissues.

Antigens

- Antigens are commonly proteins.
- However, complex polysaccharides, lipopolysaccharides, polypeptides, some artificial polymeric compounds have antigenic properties as well.

Properties of antigen:

- **Foreigness**– the main feature of antigen. An antigen must be a foreign substance for organism.
- However, antigenic determinants of genetically nonrelated animals or biopolymers may have similarities. They are called ***cross antigens***.
- The antigens of some microorganisms are not recognized by immune factors because they are similar to the antigens of the human body. This phenomenon known as antigenic mimicry.

Xeno-, allo-, izoantigens

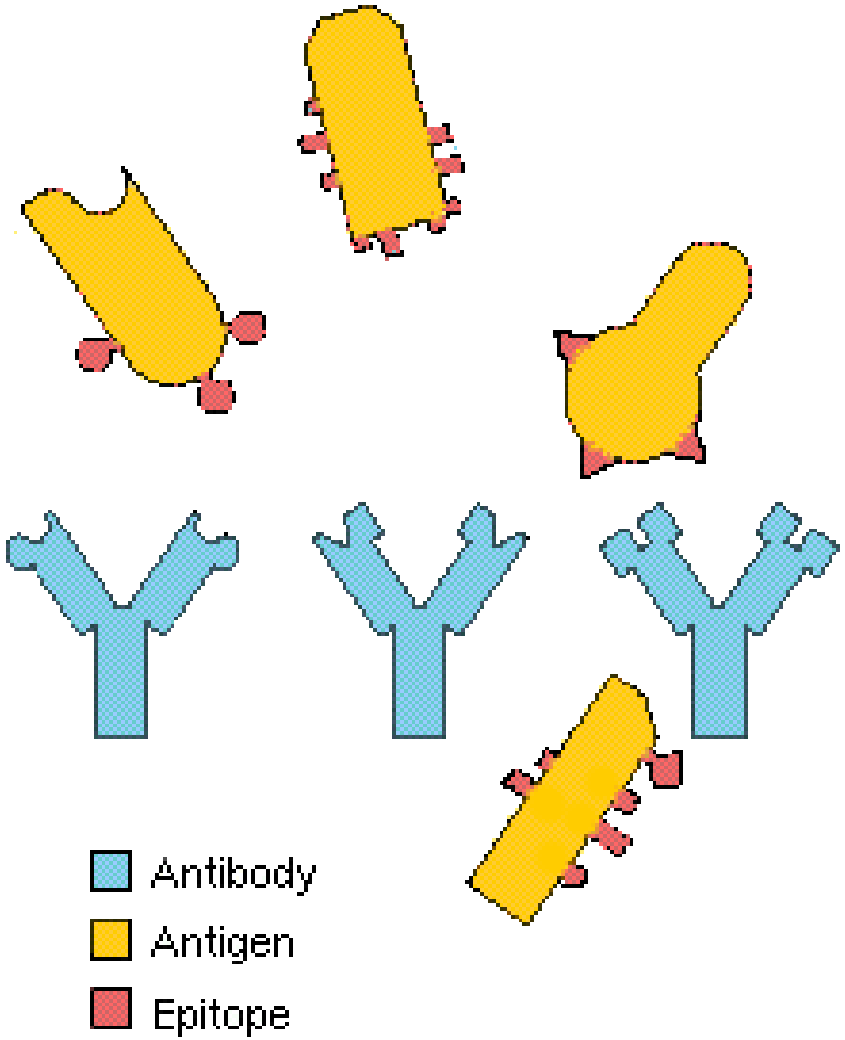
- ***Xenoantigens,* or *heterophil antigens*** – are the same for organisms of same genera,species.
- ***Allogens,* or *group antigens*** are the same for genetically different same species. Based on alloantigens the population of organisms can be grouped to different groups. Exp., blood group antigens.
- ***Isogenous,* or *species antigens*** are the same only for genetically identical organisms,exp.

  Siamese twins, inbreeding animals, genetic clones.

Properties of antigen:

- **Antigenicity**– ability to induceantibody production.
- Only specific sites of antigen molecule called antigen determinants or epitopes provide antigenicity by inducing antibody productionand binding with them.
- Each antigen has one or more antigenic determinants. The majority of antigens have many epitopes in other words they are multivalent.

Epitopes



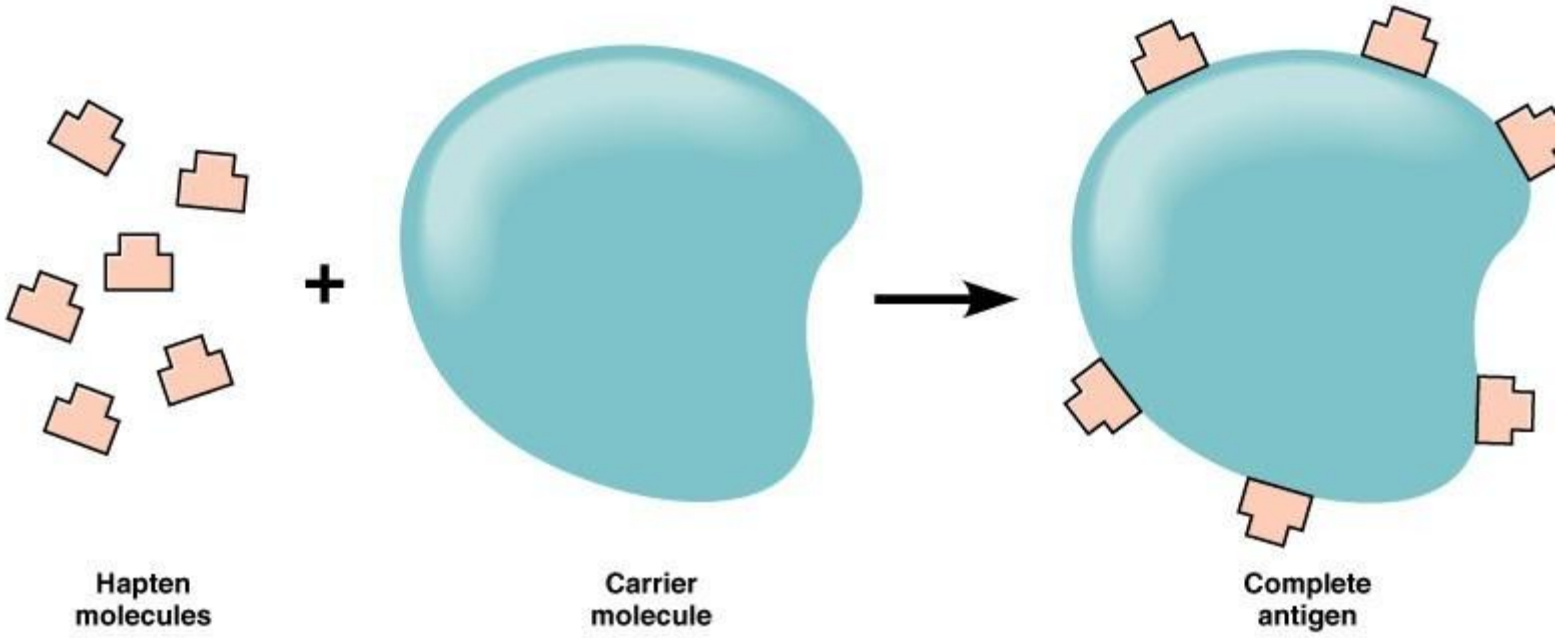
Properties of antigens:

- **Immunogenicity** – ability of antigen to form immunity.
- Immunogenicity depends on molecular structure of antigen and reactivity of macroorganism.
- Despite similarities antigenicity and immunogenicity they are different phenomenons. For example, bacterial dysentery agents have high antigenicity, however they do not form strong immunity, ie they have weak immunogenicity.

Haptens

- ***Haptens***, or inncomplete antigens have antigenicity and weak immunogenicity.
- They are small nonproteinic molecules that elicit an immune response only when attached to a large carrier such as a protein

Haptens



Properties of antigens

- **Specificity** – ability of antigen to elicit specific immune response.
- Interactions between antigens and antibodies have high specificity. This feature is used in diagnosis of microorganisms in diagnostic laboratories.
- Strength of antibody-antigen connection – affinity vary in proportion with the similarity of their binding sites. Antigens differ in their affinity.

Immunogens, tolerogens and allergens

- ***Immunogens*** cause productive infection accompanied with release of immunity factors (antibodies, antigen reactive lymphocyte clones).

  -*T-dependent antigens*

  -*T-independent antigens*

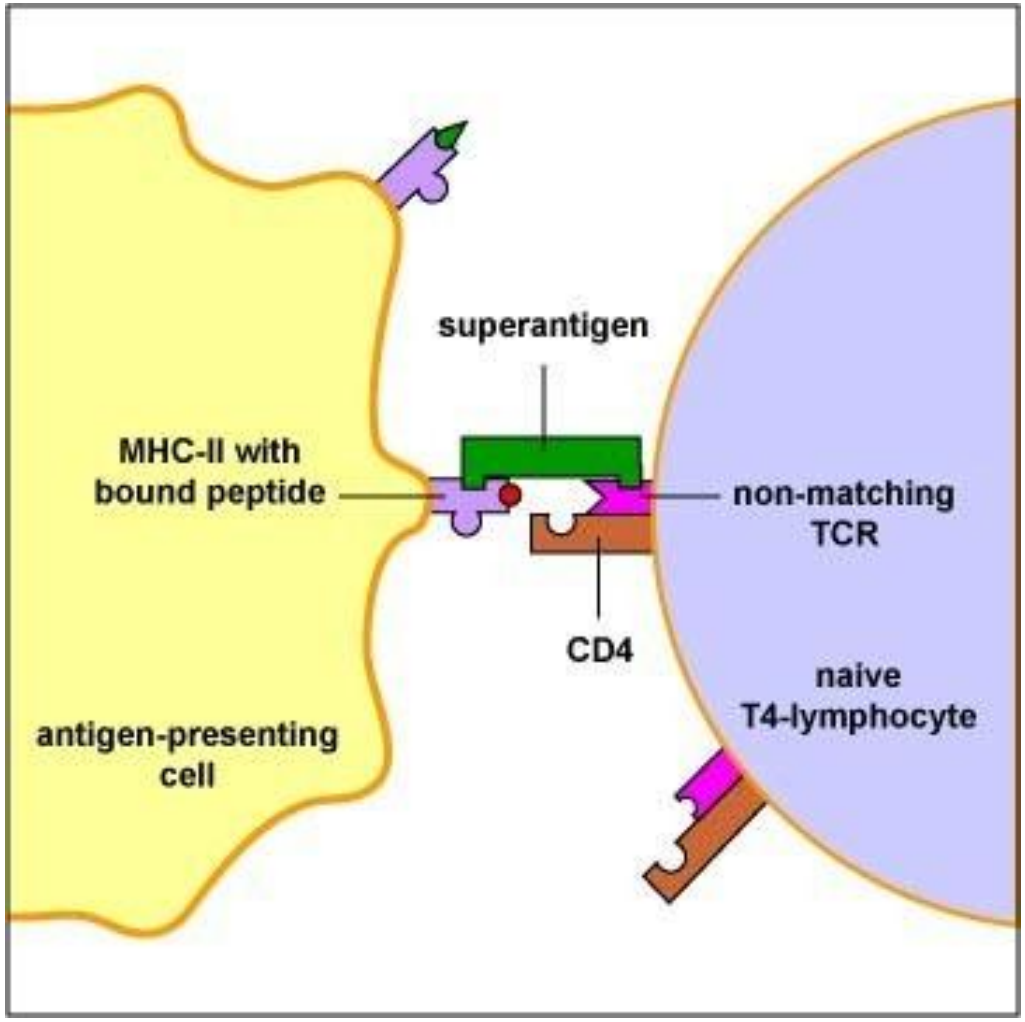
- ***Tolerogens*** – induce tolerancy or areactivity in macroorganism. Tolerogenic molecules are characterized by high dispersion due to their monomerism, small molecular weight, high density of epitopes.

- ***Allergens*** do not differ from immunogens and cause immediate or delayed hipersensitivity reactions.

Superantigens

- Some antigens can activate T-helpers without APC and T-helper cooperation.
- These molecules called ***superantigen***s can bind to MHC II-TCR complex and form false signal.

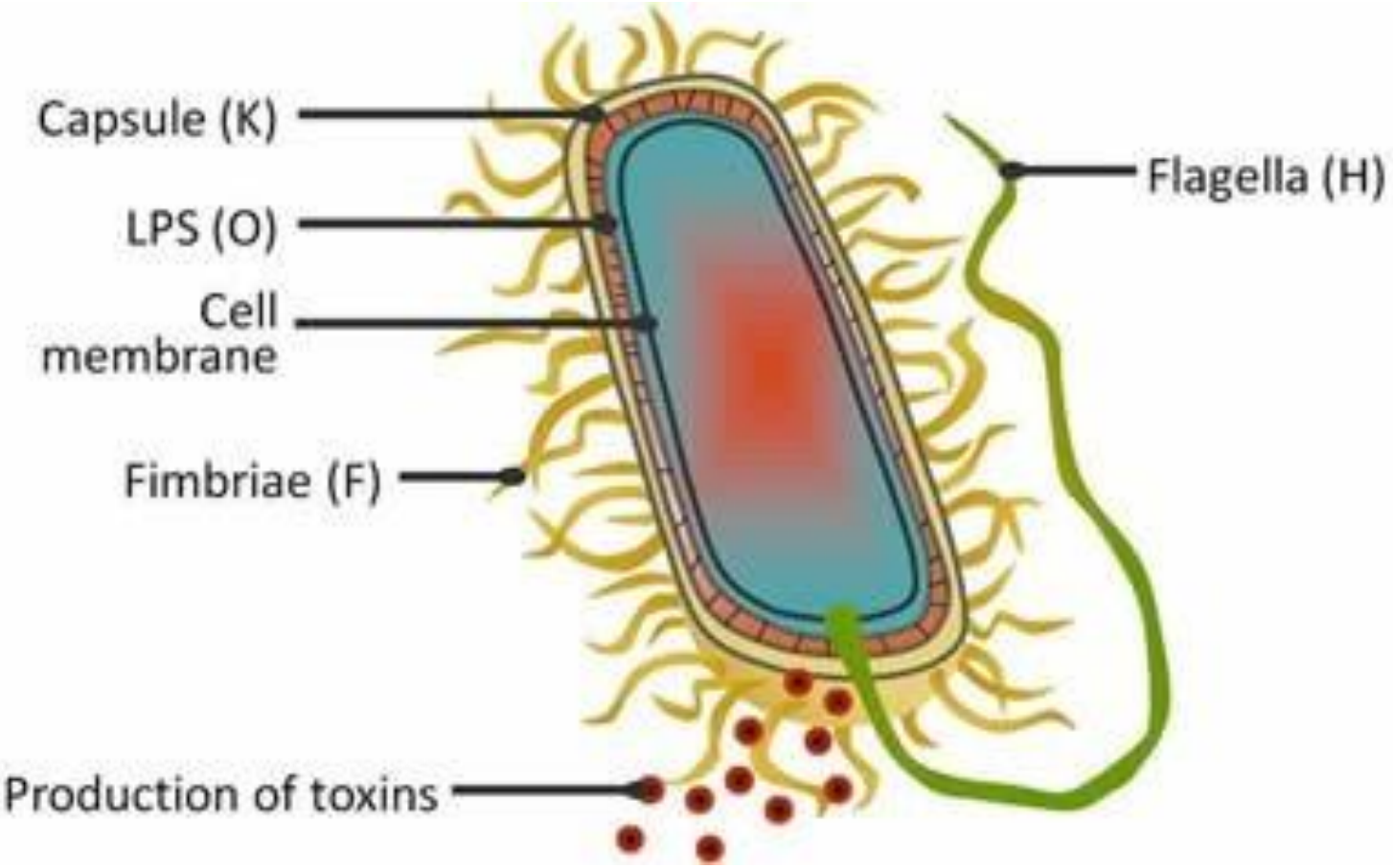
Superantigens



Microorganism antigens

- Bacterial antigens
- *Flagella antigen, or H-antigen*
- *Somatic, or O-antigen*
- *Capsule*, or *K-antigen*
- *virulency antigen*, or *Vi-antigen*
- *Exotoxins, enzymes*
- Viral antigens
- *Virus specific antigens*

Bacterial antigens



Human organism antigens

- *Erythrocyte antigens*
- *ABO system antigens*
- *rhezus-antigens*
- *Major Hystocompatibility Complex –MHC (Human Leukocyte Antigen* - HLA) antigens
- 2 types of MHC antigens.
- I.class MHC exist in all nucleated cells,
- II.class MHC exist commonly in immune competent cells.

Hystocompatibility antigens

- Tissue compatibility antigens are found on the membranes of all cells in the body.
- Most of them belong to the *Main Hystocompatibility Complex* (*MHC*) antigens.

MHC

Human MHC antigen is called HLA as it first was described in leucocytes (*Human Leukocyte Antigen*).

HLA synthesis is provided by genes located in the short arm of the 6th human chromosome. Three of these genes - HLA-A, HLA-B and HLA-C- encode MHC class I proteins.

Some HLA-D loci encode class II MHC proteins (DP, DQ and DR).

Locus III is located between I and II loci. The genes that encode the two components of the complement (C2 and C4) are located in this locus.

MHC

- Thus, there are two main classes of MHC molecules. Class I MHC is expressed in all nuclear cells, and Class Ⅱ MHC is mainly expressed onthe surface of immunocompetent cells.

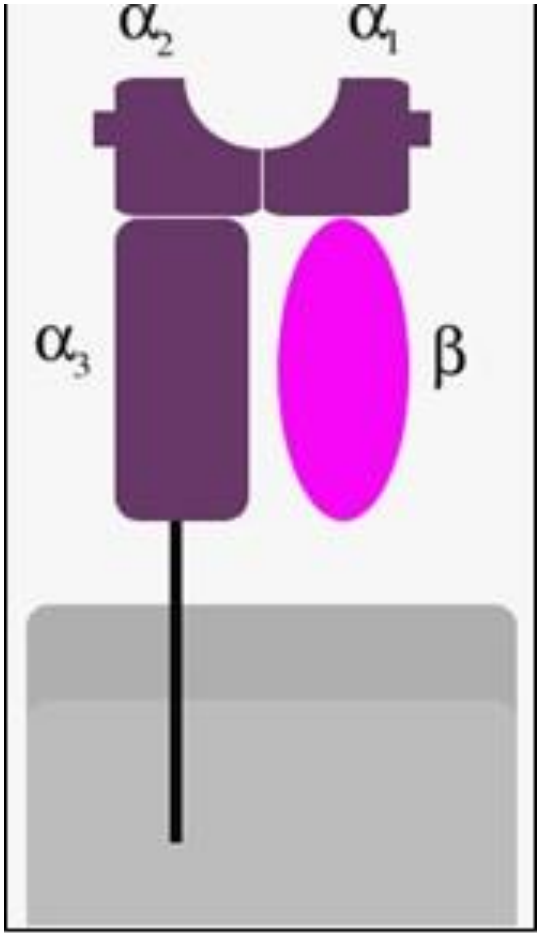
  There are no individuals with the same MHC antigen in the entire human population, in other words, all people differ in theseantigens.

- However, the exception is single-egg twins, as well as genetic clones. Therefore, the compatibility of these antigens in tissue transplantation (relative compatibility), is taken into account.

MHC structure and fumctions

- MHC antigens are glycoproteins located on cell membrane
- Some MHC fragments have homologous with immunoglobulins structure

I class MHC glycoproteins located in all nucleated cells



I class MHC are unique for each individual, biological passport of organism and a "native" markers of immune competent cells.

Viral infections and mutations alter the structureof MHC class I.

- Modified MHC I are cause activation of T - killers ($CD8^+$ lymphocytes).
- Thus, cells with altered MHC I are recognized as foreign cells and destroyed.

II class MHC

II class MHC proteins are glycoproteins and located on macrophage, T-helper, B-lymphocytes, spleen, dendritic cells surface

II class MHC differ structurally and functionally from I class MHC.

- I I class MHC are expressed only in specific cell (especially immune competent cells) surfaces.
- I Iclass MHC contain peptides obtained by endocytosis and not synthesized in cells, for exp. Viral antigens.

IIclass MHC participate in immune response induction

- This process has several steps:
- Fragments of the antigen molecule are expressed on the surface of APC (dendritic cell, macrophage etc.) in the form of a complex "class II MHC + antigen".
- This complex is recognized and analyzed by T-helpers (CD4 + lymphocytes).
- When the peptide in a Class II MHC is detected, the T-helper begins to synthesize the appropriate cytokines and the specific immune response mechanism begins to work.

CD-antigens

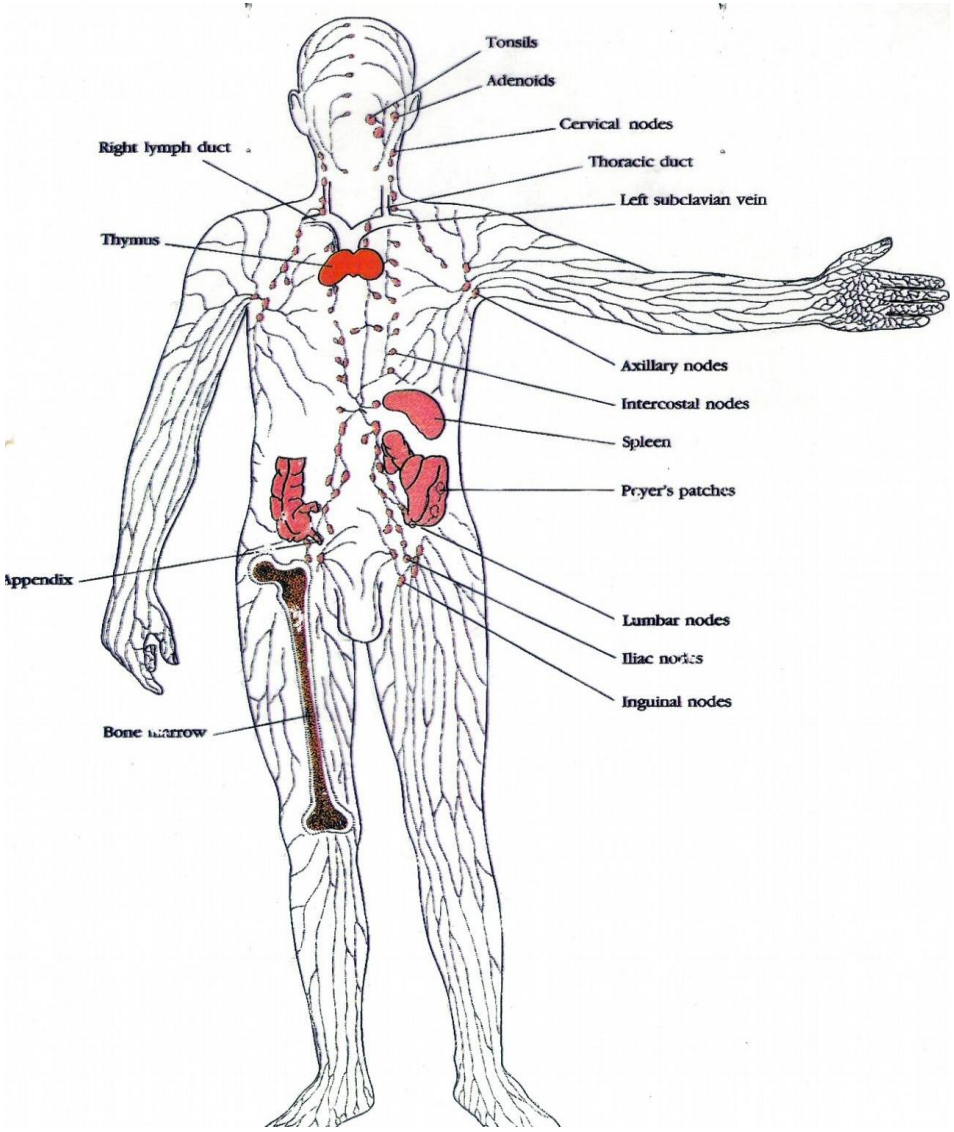
- Cell membranes have morfofunctionally identical group antigens called markers. Markers of immune competent cells are well studied.
- These antigens are called ***CD-antigens (cell differentiation antigen)***. They are structurally glycoproteins and some have immunoglobuline nature.

Immune system of organism

Cells, tissues and organs developing response to genetically foreign substances – immune system of organism. Immune system has three main features:

- It is spread throughout the body;
- It has cells circulating in blood, lymphatic system;
- The immune system has a unique ability to produce antibody molecules, immunoglobulins, which have a very high specificity against various antigens that are genetically foreign.

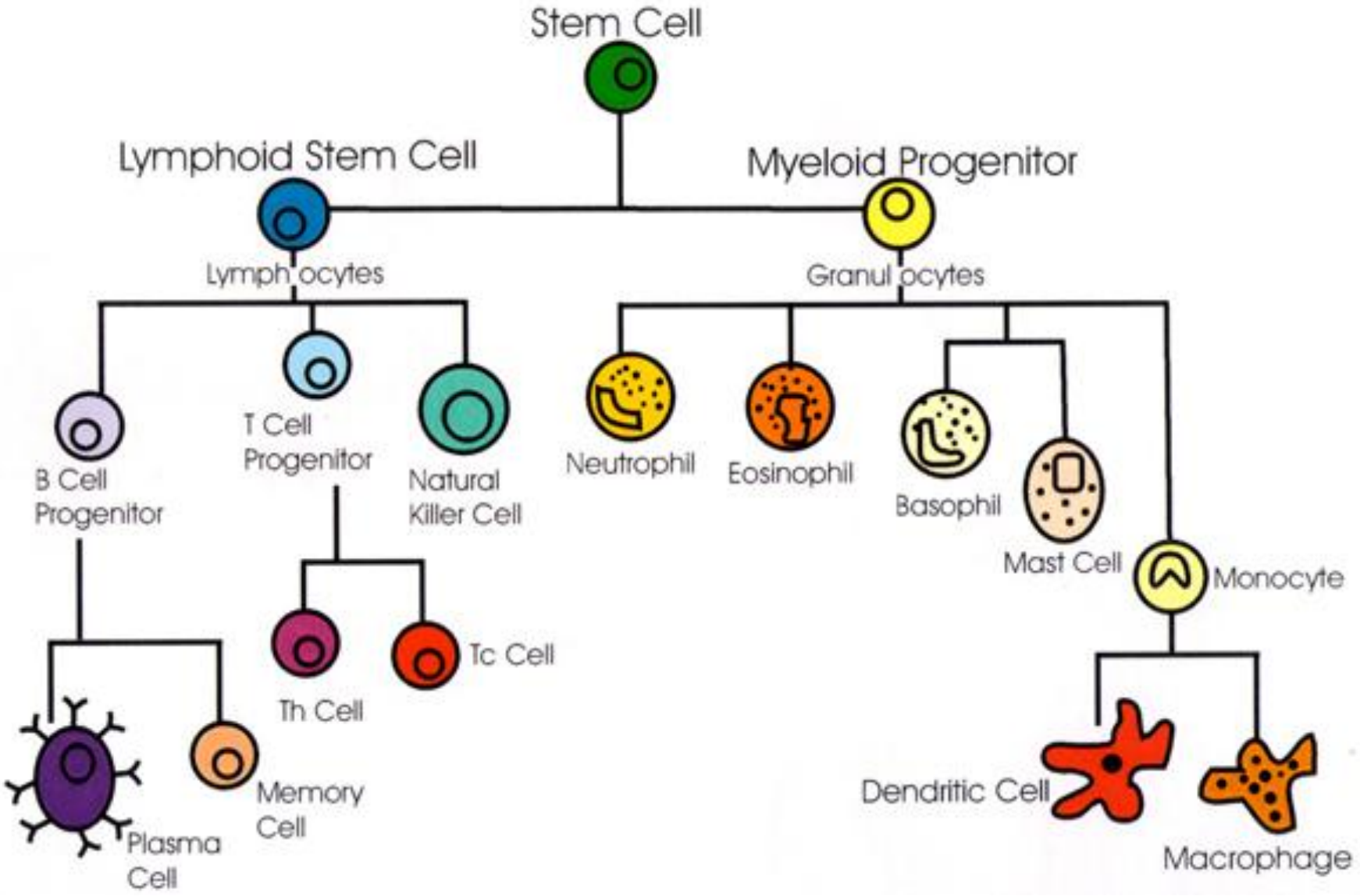
Immune system



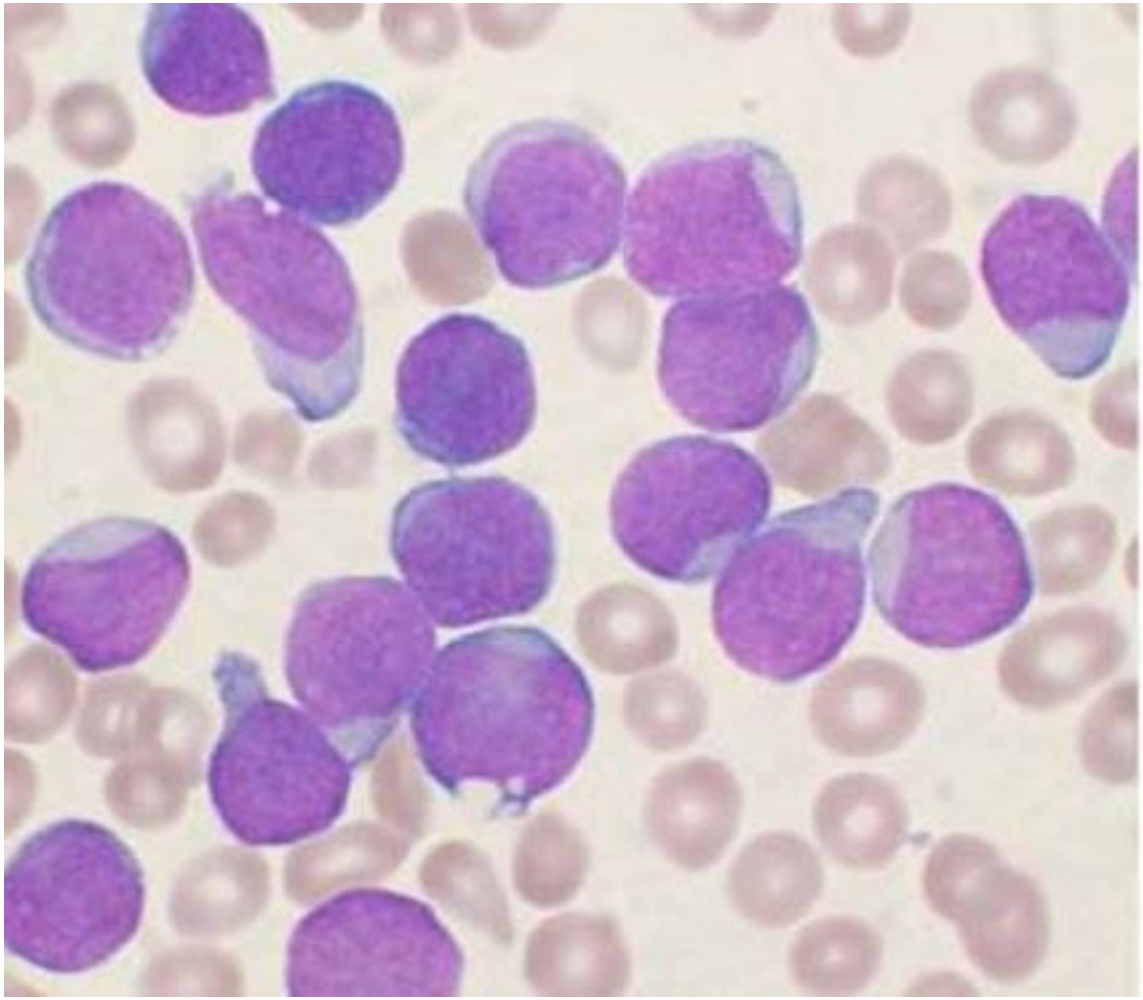
Organs of immune system

- **Central organs of immune system-** support creation and selection of immune cells
- bone marrow, thymus
- **Periferic organs** – control genetic stability of organism
- spleen, lymphatic nodes and follicles

Cells of the Immune System



Immune system cells - lymphocytes



Lymphocytes

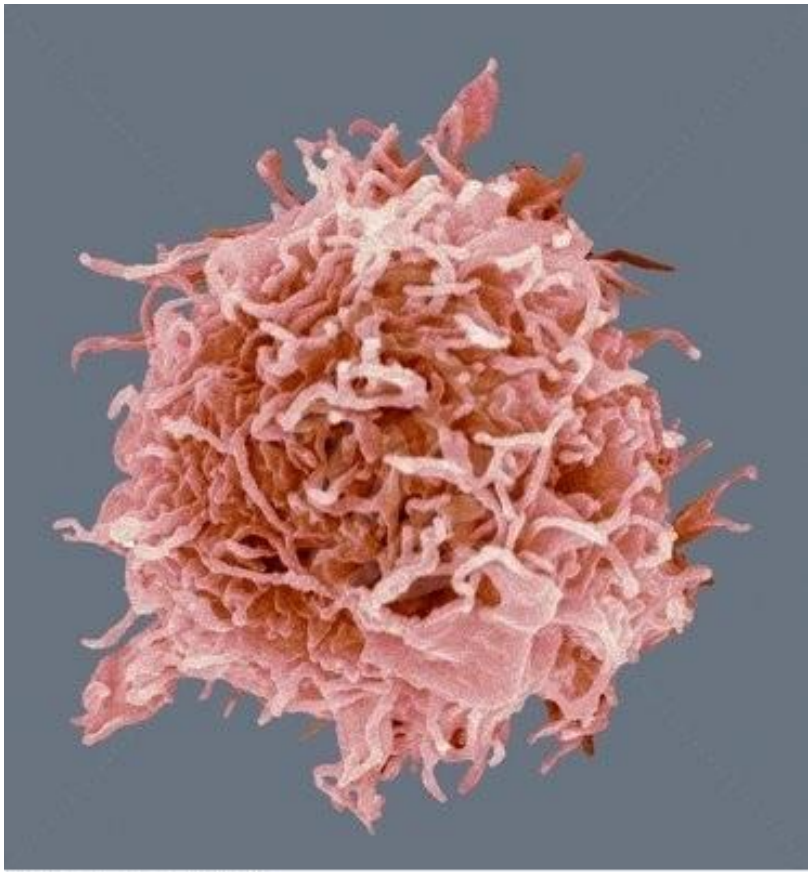
Mature lymphocytes have two subpopulations:

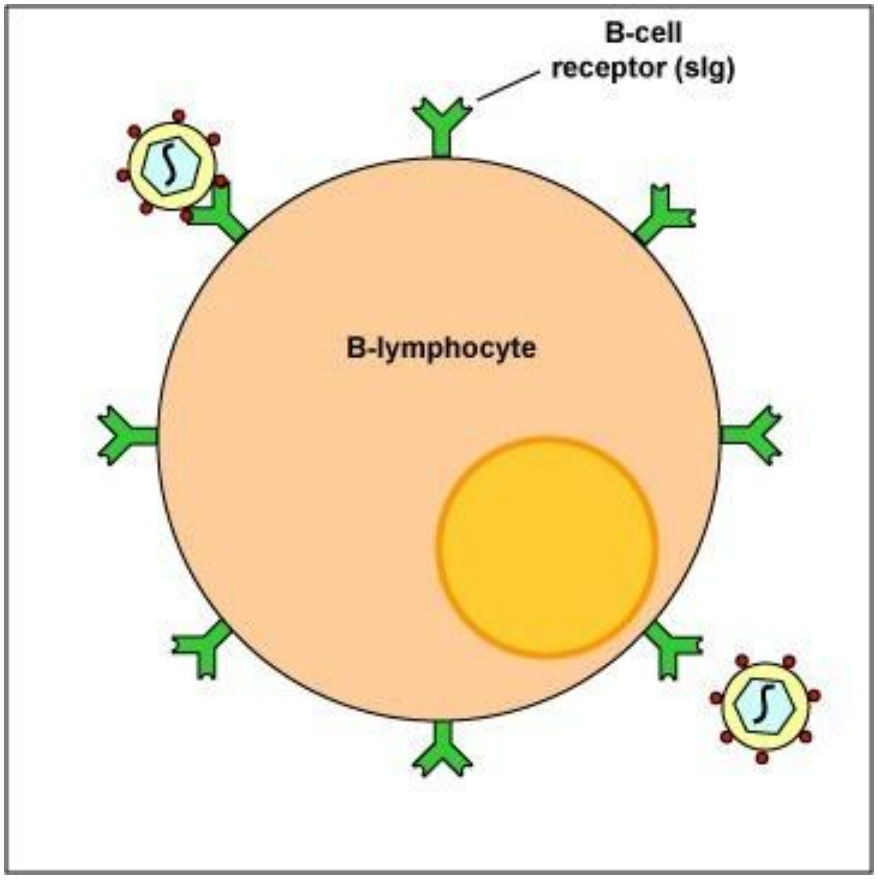
- **B -** lymphocytes
- **T –** lymphocytes
- **O –** lymphocytes

B-lymphocytes and plasmocytes

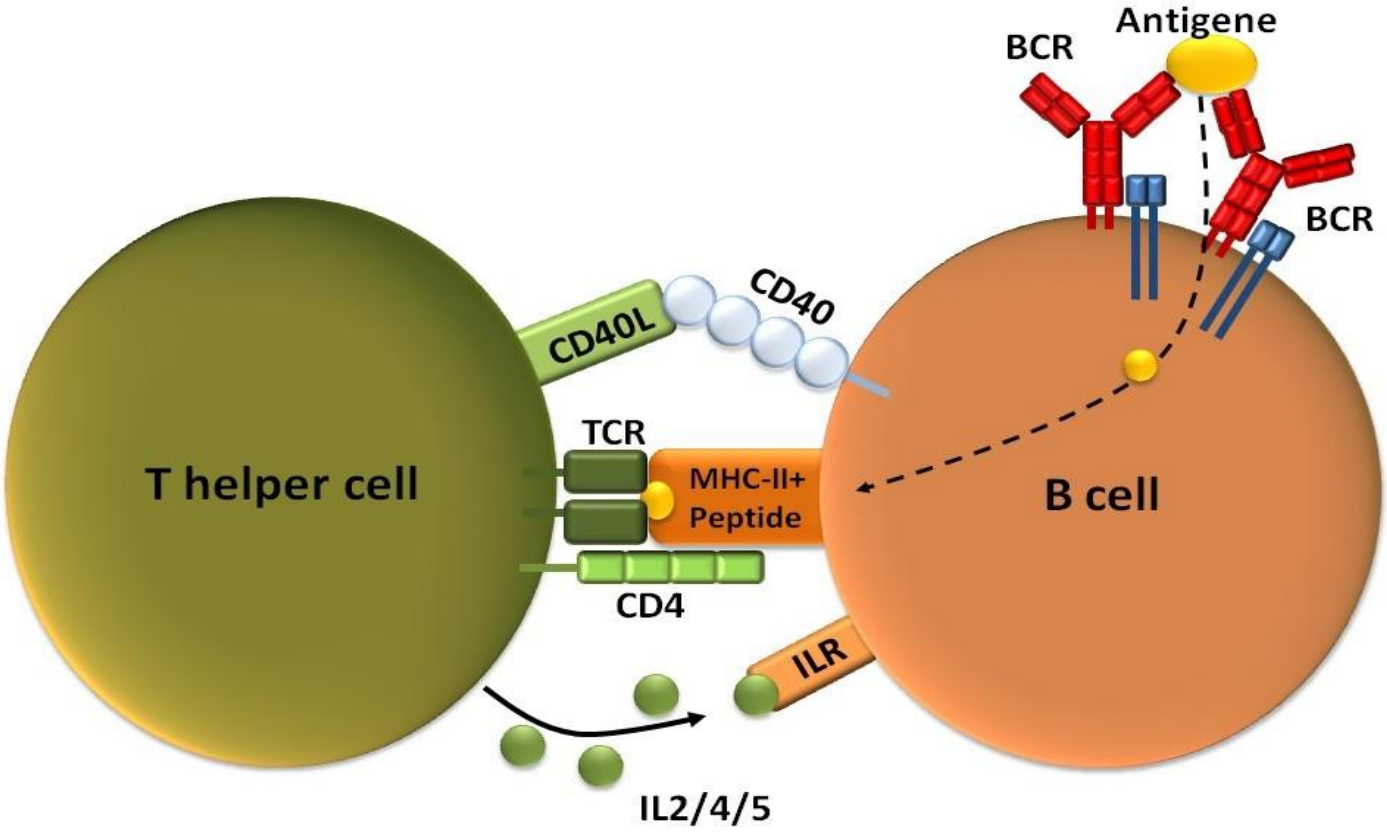
- Create humoral immunity by synthesis of antibodies
- Participate in development of immunological memory
- Participate in immediate type immune responses

B-lymphocytes





B-lymphocytes recognize antigen in cooperation with T-helpers



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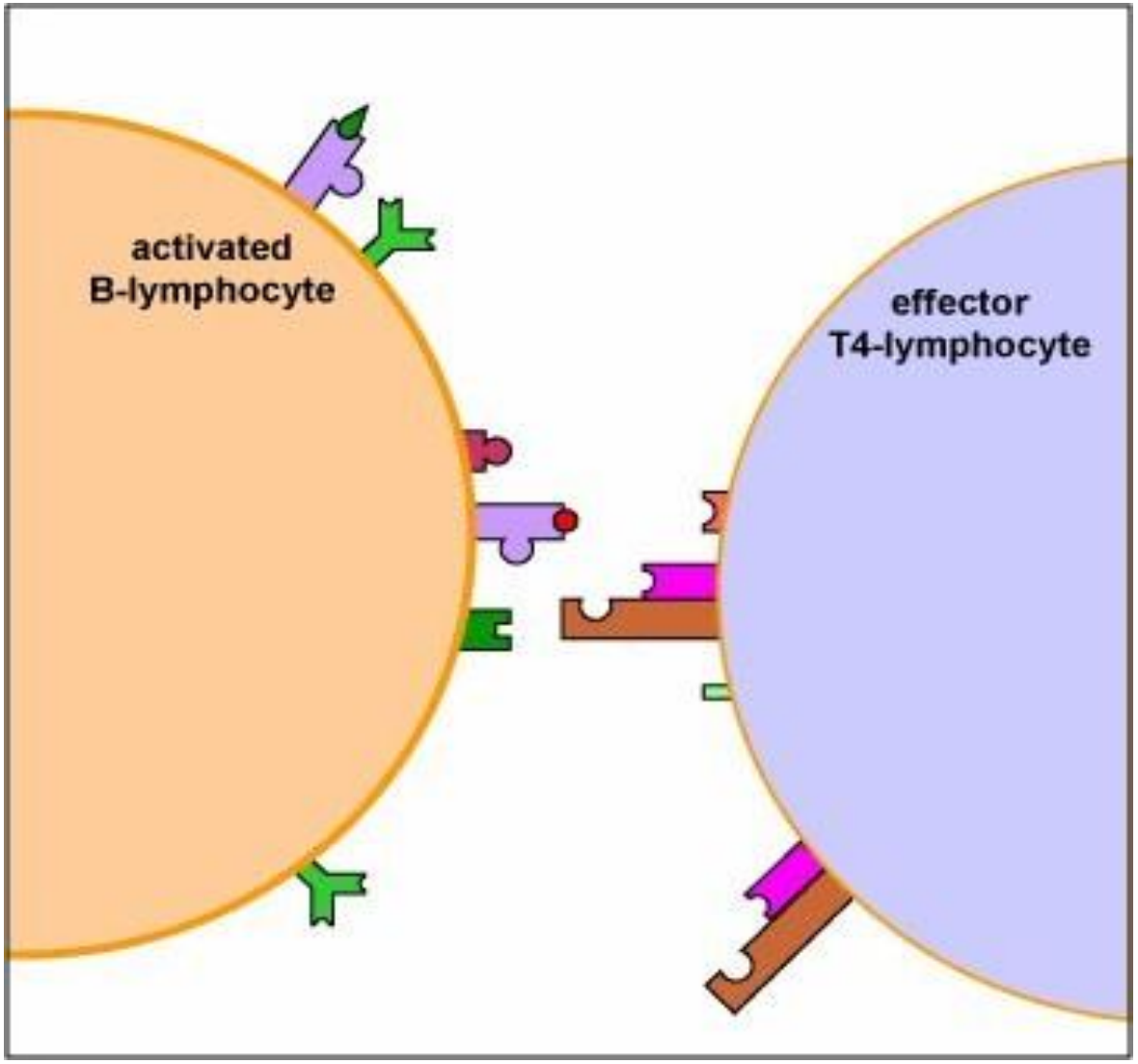


T-lymphocytes

- **T-helpers (CD4)**

Recognize antigens with help of antigen presenting cells and activate other immune cells

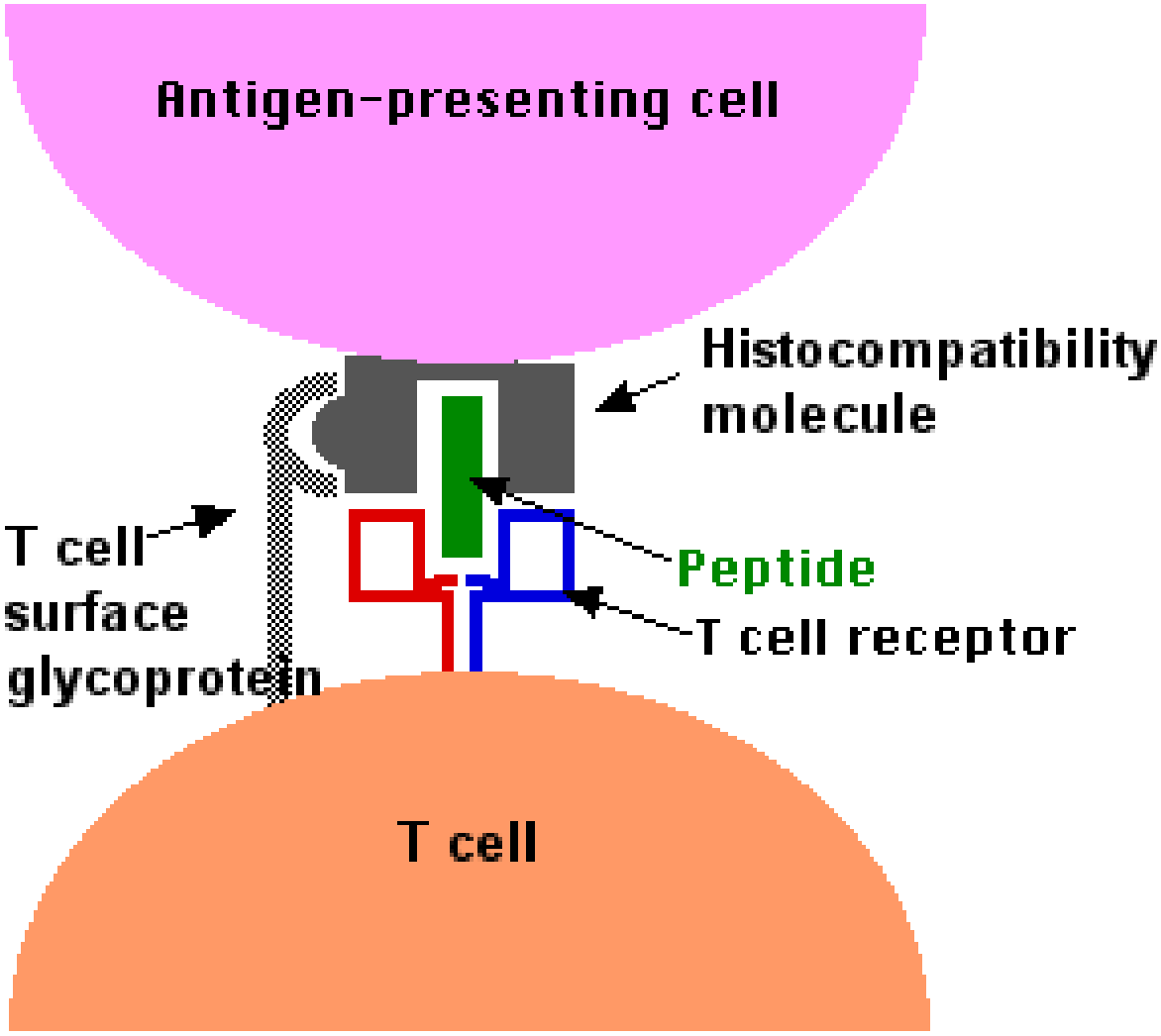
- **T-killers (CD8)**

kill target cells by antibody independent cytotoxicity

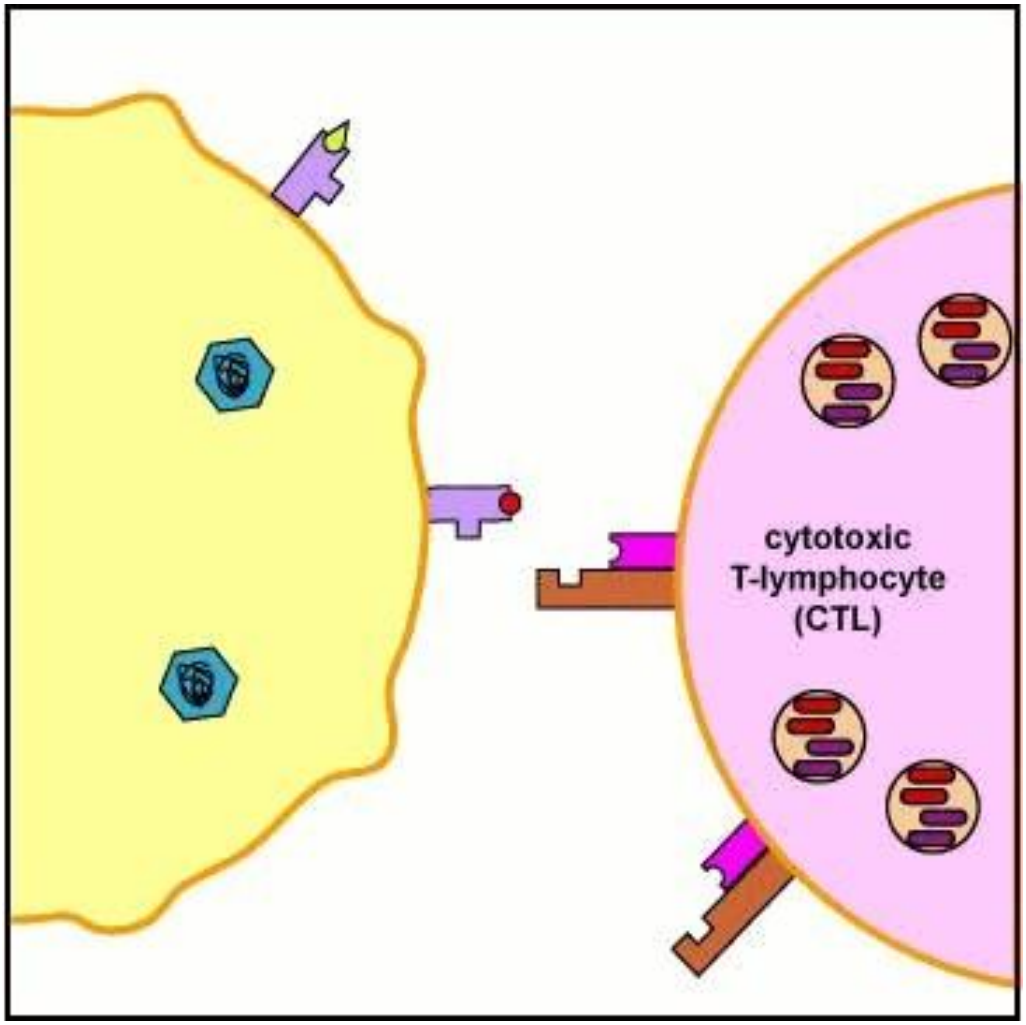
- **T-supressors**

weaken immune response, thus playing immune regulatory role

T-lymphocytes recognize antigens presented by macrophages



T-lymphocytes (T-killers) destroy target cells by antibody independent cytotoxicity

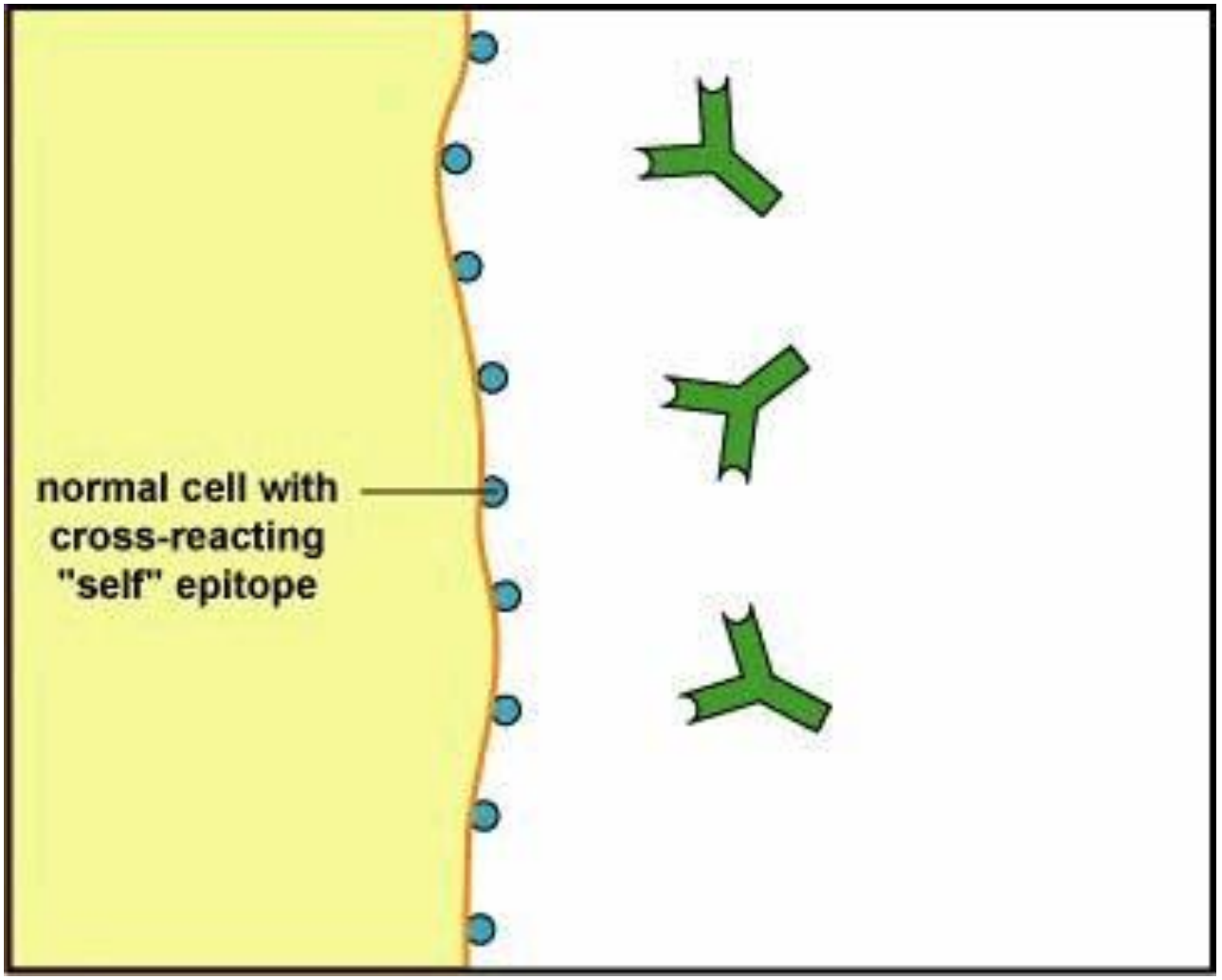


NK-cells
(eng. «natural killer»)

- The main defensive cells against intracellular parasites and genetically foreign cells (tumour cells)
- Act independently from specific immunity
- Destroy target cells by antibody dependent and independent cell cytotoxicity

NK-cells attack tumour cells

NK-cells action on target cell



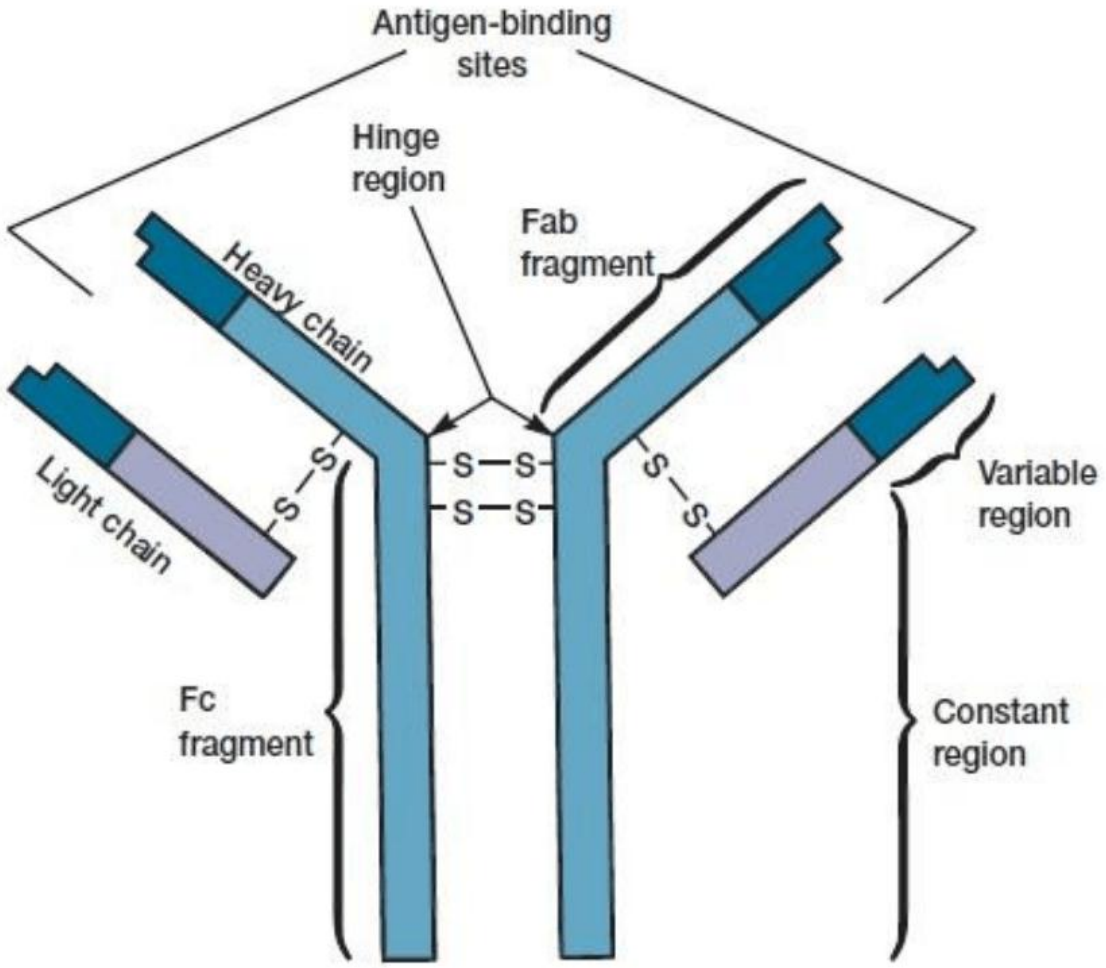
Immunoglobulins or antibodies

- 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 I I proteins
- Th- Lymphocytes produce - IL2 (T-cells growth factor), IL4 (B-lymphocytes growth factor) and IL5 (B-lymphocytes differentiation factor). These cytokines active antigen specific B-lymphocytes. Activated B-lymphocytes proliferate and differentiate into plasma cells producing immunoglobulins (antibodies).

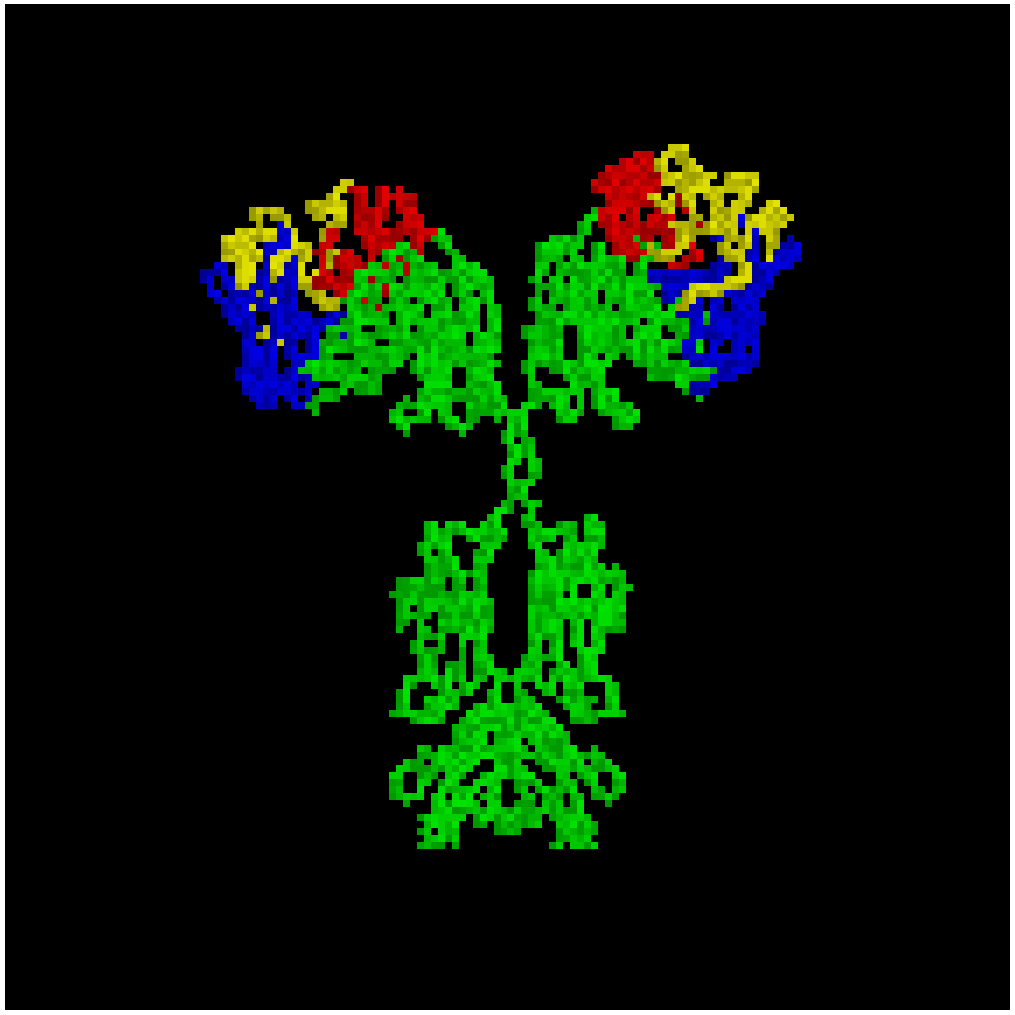
Immunoglobulins

- Immunoglobulines (Ig) are gamma-globulin fraction protein.
- The Ig monomer is composed of two **light (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).**

Antibody or Immunoglobulin



Immunoglobulin model



Immunoglobulin structure

- L- and H-chain terminal regions have 3 extremely 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 non-covalent electrostatic, van-der-vaals, hydrogen and hydrophobic bonds.

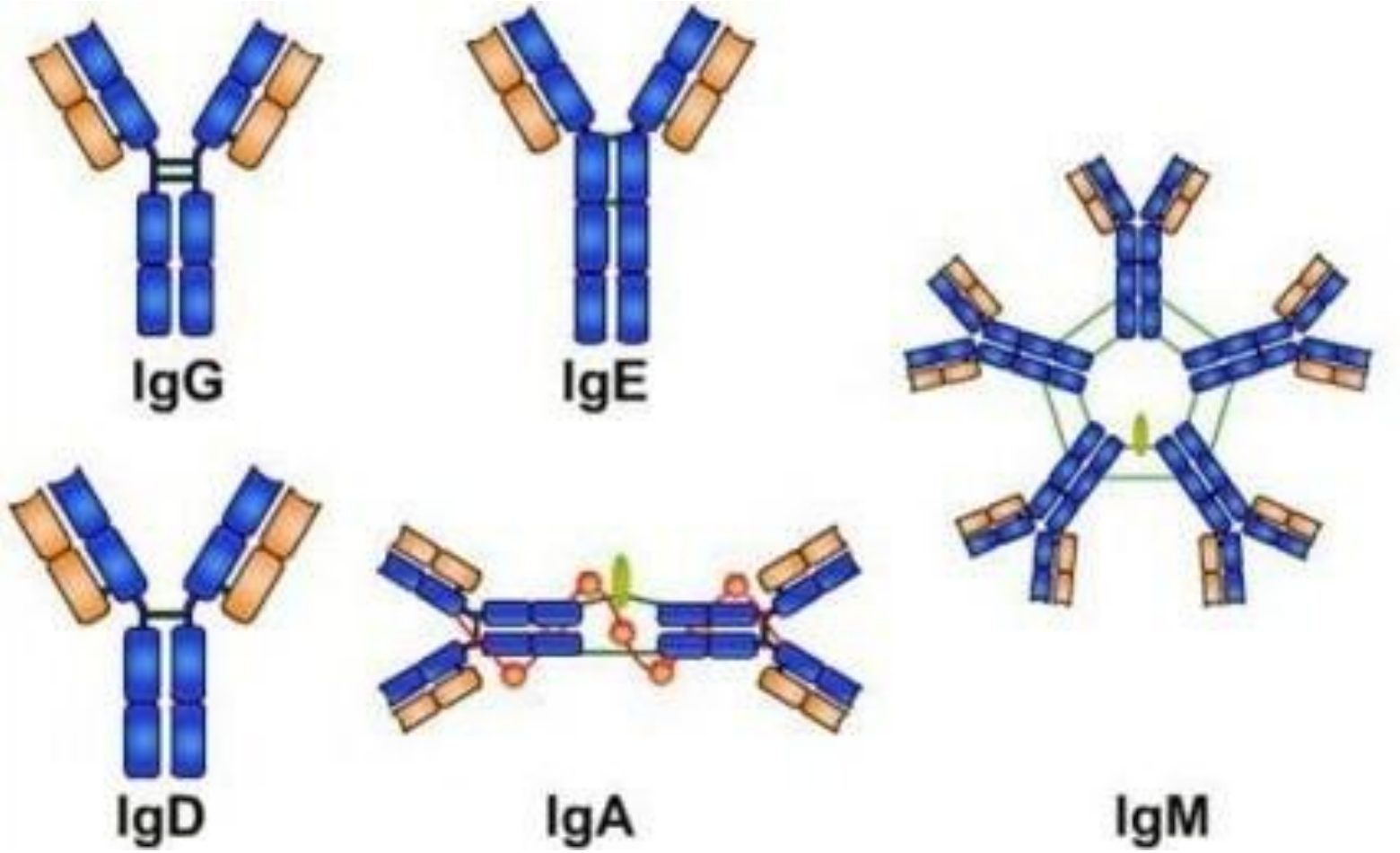
Immunoglobulin structure

- H and L chains have constant domens called ***Fc*-fraqments (*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 1Fc fragments.

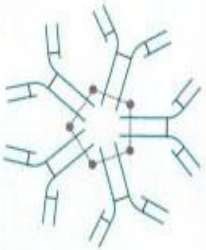
Immunoglobulin classes

- 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.

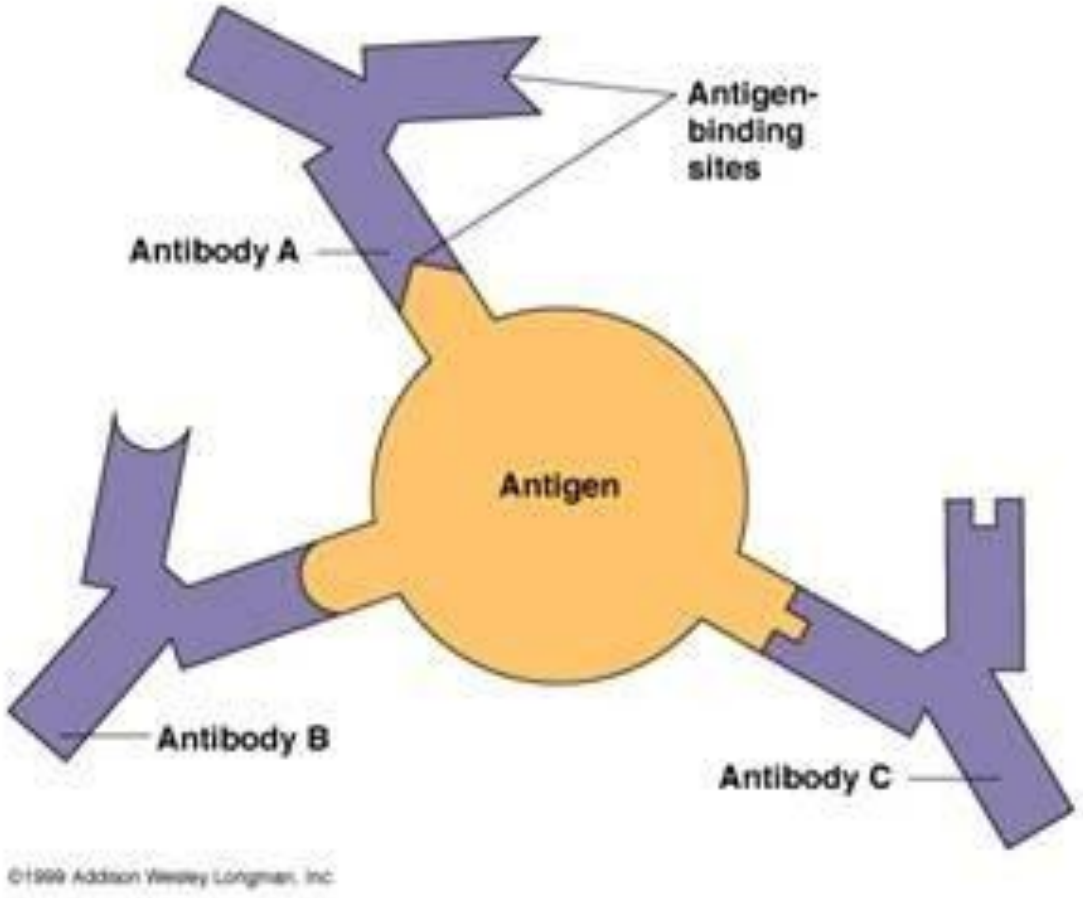
Immunoglobulin classes

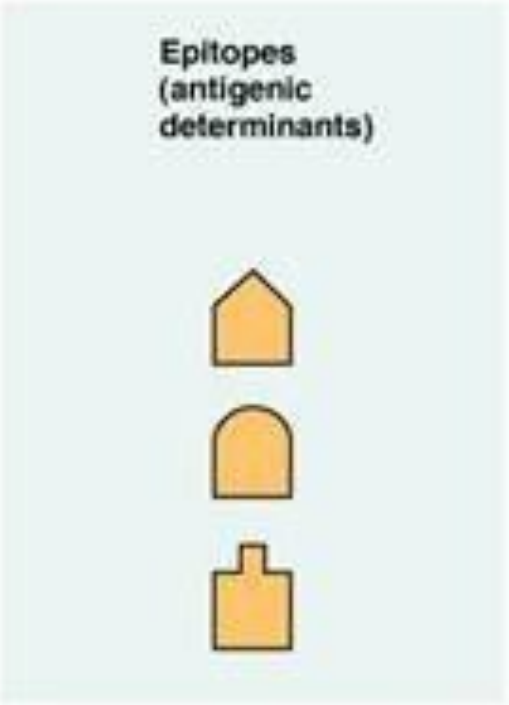


Immunoglobulin classes

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

Specificity of antibodies

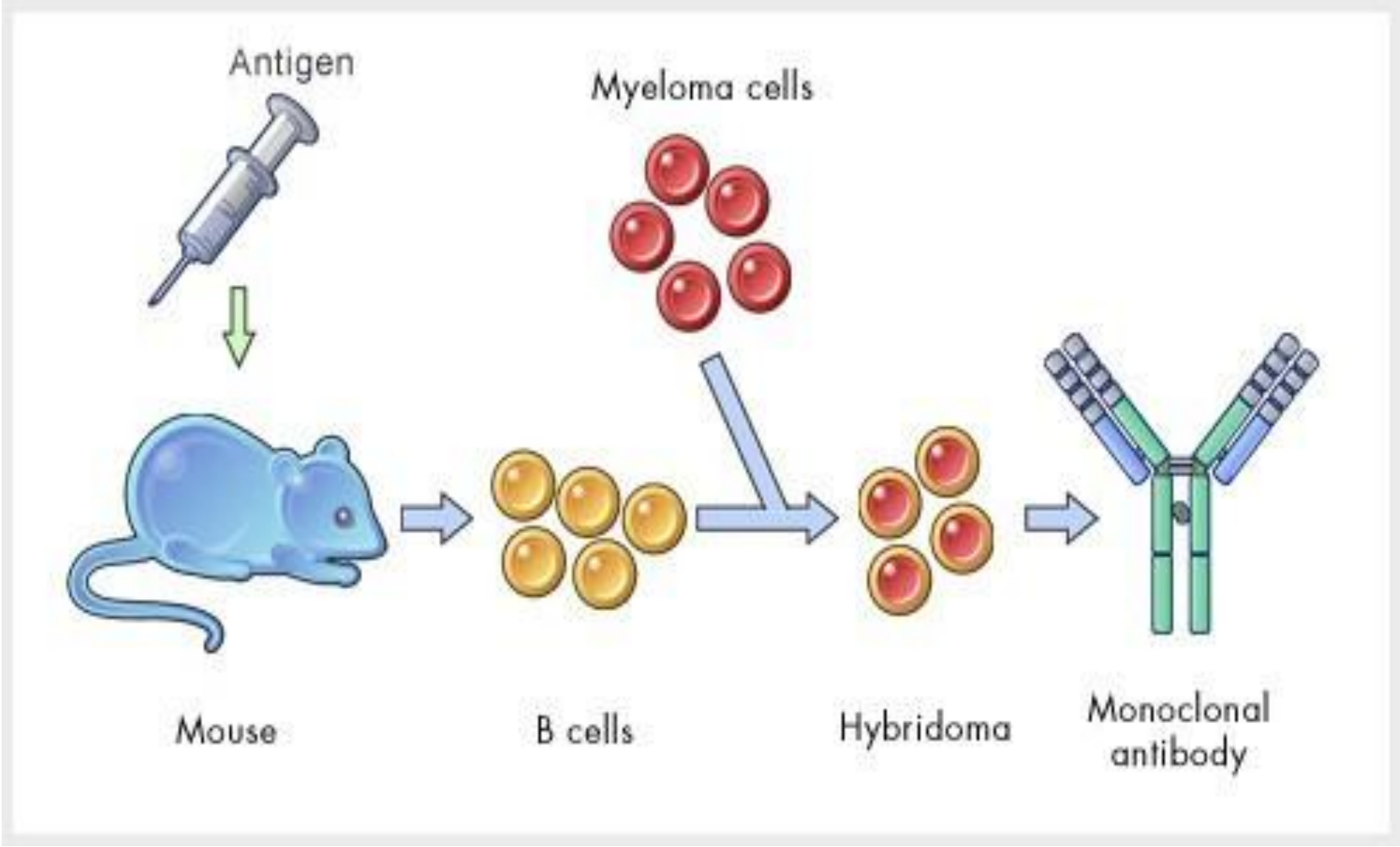




Diversity of antibodies

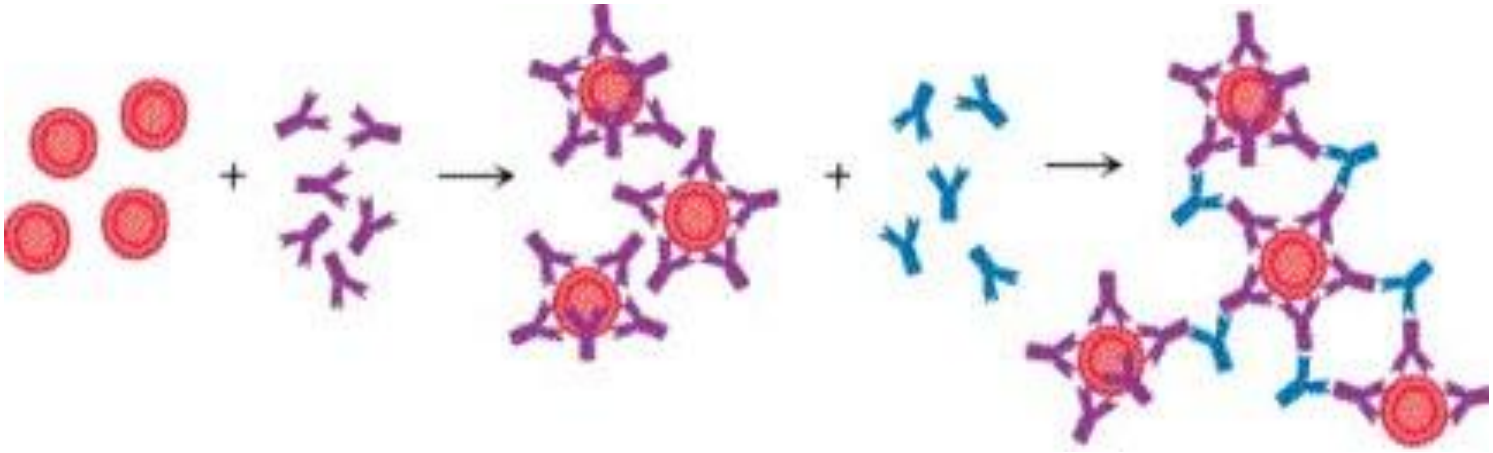
- Normal, or natural antibodies
- Receptor immunoglobulins
- Policlonal antibodies
- Monoclonal antibodies – D.Keller and T.Milstein in 1975 obtained hybridoma by synthesis of antibody and attaching it to B- lymphocyte myeloma cells.
- Non-complete, blocking antibodies

Obtaining monoclonal antibodies



Blocking antibodies

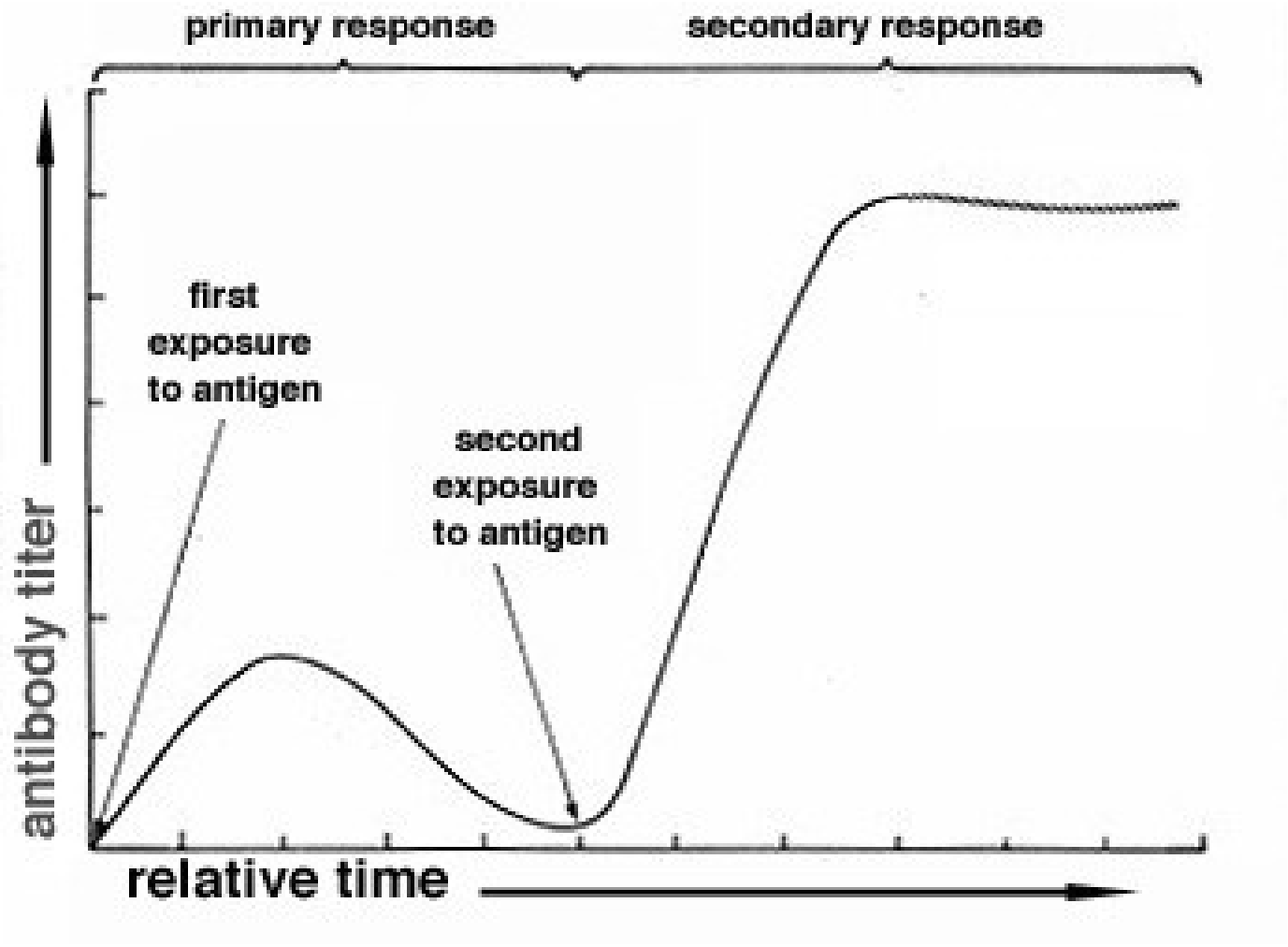
- Sometimes, due to the absence of one of the active centers in the Ig molecule, they combine with the antigen only with one center Thus, there is no formation of large aggregates. Therefore, such antibodies are called incomplete or blocking antibodies.
- Incomplete antibodies are detected by ***Coombs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



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.

Immune diagnostics

- Diagnosis of diseases by application of immune reactions is widely used in practical medicine. The most commonly used reactions in immunodiagnostics are based on the specific interaction of antibodies and antigens.
- It is known that specific antibodies are formed in the blood serum against antigens that enter the body. These antibodies have the ability to bind specifically to antigens not only in the body (in vivo) but also outside the body (in vitro).
- Because the interaction between antibodies and antigens is so specific, it is possible to identify an unknown antibody based on a known antigen, or vice versa.
- These reactions are called **serological reactions** because of the use of serum containing antibodies.

Application of serological reactions

2 ways of application is possible:

- Identification of antigens, microorganisms and their toxins using known antibodies by serological reaction.
- These antibodies are used in form of ***diagnostic immune serum***. Identification of microorganism by known immune serum is called ***serological identification.***

Application of serological reactions

- Detection of antibodies in serological reactions is performed by usage ofknown antigens – ***diagnosticum***.
- Reference strains or their antigens are used as diagnosticum. Unknown antibodies are commonly examined in patient plasma.
- Presence of antibody titer indicates infection, thus method is called ***serological diagnostics***.

Application of serological reactions

- The result of serological reaction is evaluated on basis of formation of *antigen-antibody* complex. Positive serological reaction is accompanied by formation of this complex.
- Depending on mechanism, ingredients, features of antigen-antibody complex different reactions are distinguished:

  - **Simple** (2 components)

  - **Complex** (3 and more components).
- **Agglutination, precipitation, neutralization, complement fixation, reactions with labeled antibody or antigen** are used in immune diagnostics.

Serological reaction stages

There are two phases in any serological reaction:
Specific phase - occurs quickly. Antibodies (paratop) combine with the corresponding antigen (epitop). Participates in the formation of the Ag +Ab complex:
- Coulomb force
- Van der Vaals force
- Hydrogen bonds.

No visible change is observedin this phase.
Non-specific phase - occurs slowly. The resulting Ag+Ab complex occurs in the presence of additional non-specific environmental factors (electrolyte, pH, etc.) with a visible reaction (sedimentation, etc.).
In the presence of the electrolyte, decrease of electric charge and solubility results in a visible conglomerate formation (e.g., agglutination).

Agglutination phenomenon