Lecture VI

Acquired (specific) immunity and types.Antigens, their types. Antigenic structure of microorganisms. Basic principles of human immune system. Immune competent cell.

Immune response reactions. Production of antibodies. Immunoglobulins and their classes.

Immune system

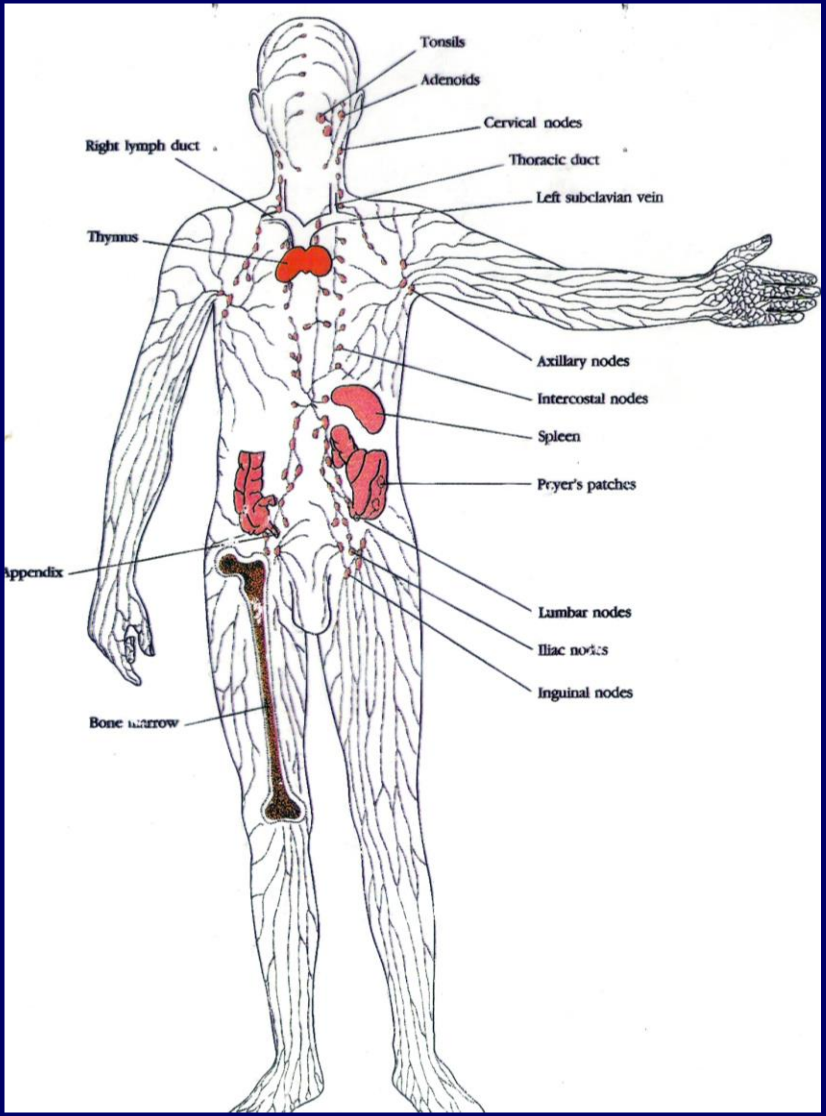
- The body's immune system is made up of a set of cells, tissues, and organs that will carry out responses to genetic foreign substances in the body.
- The immune system is protected from bacteria, viruses, parasites, etc. Provides protection of the organism, elimination (elimination) of dead or dying, mutated cells.

Immune system

The immune system has three main features:

- **The immune system is spread throughout the body;**
- **Many of its cells are constantly circulating in the blood and lymph throughout the body;**
- **The immune system has the ability to produce immunoglobulins with extremely high specificity against various antigens.**

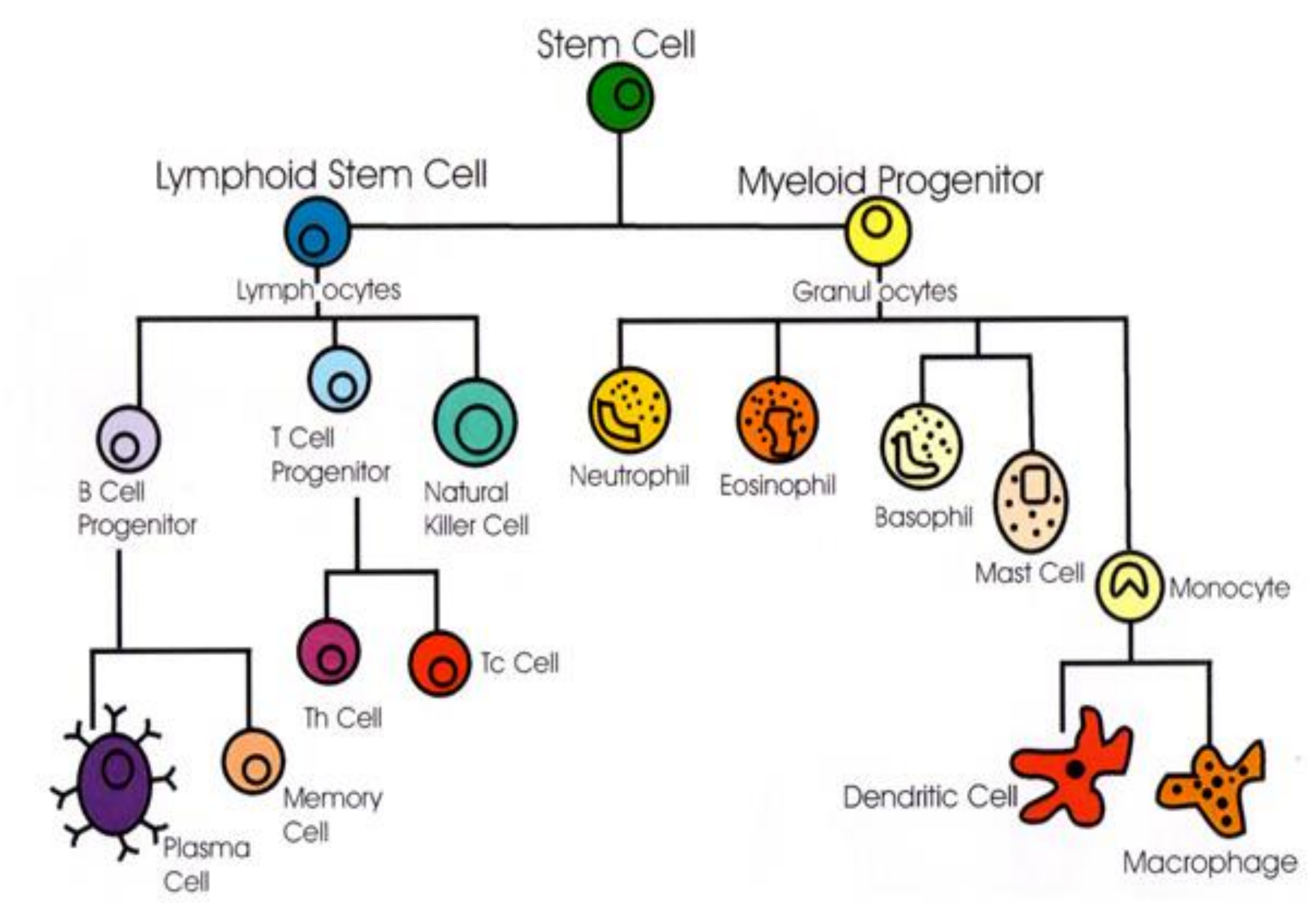
Immune system



Organs of the immune system

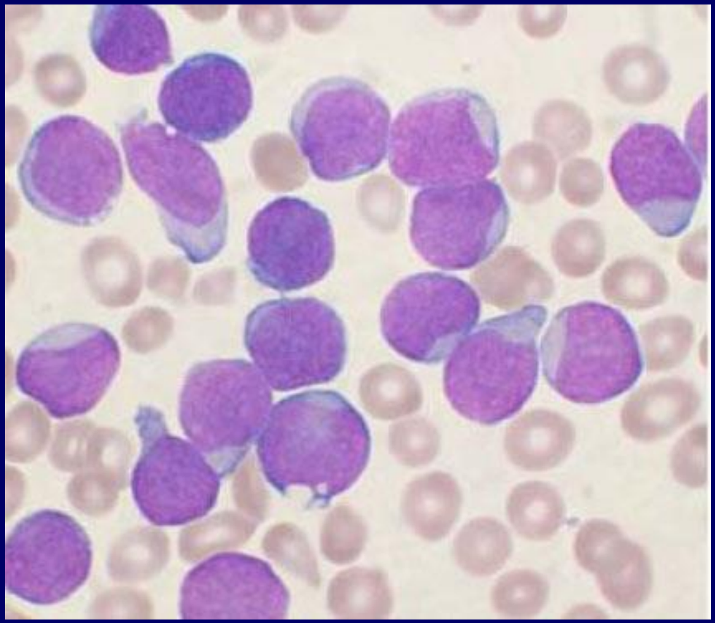
- **Central organs of the immune system - provides the formation and selection of cells of the immune system - Bone marrow, thymus**
- **Peripheral organs of the immune system - control the genetic stability of the body's internal environment - Spleen, lymph nodes, lymph follicles**

The formation of cells of the immune system



Cells of the immune system - lymphocytes

- **The main cell of the immune system is lymphocytes. Lymphocytes make up 1/3 of the total number of leukocytes.**
- **In lymphocytes, there is a thin cytoplasmic area (circle) around the nucleus in the large circle.**
- **Lymphocytes are not only present in the blood, but also make up the bulk of the tissue fluid - the lymph and lymphoid organs.**



Cells of the immune system - lymphocytes

- Lymphocytes are formed from polypotent stem cells in hematopoietic tissue.
- In mammals, the liver is considered to be the main organ of hematopoiesis in the mother's womb.
- In the postnatal period, the liver is completely deprived of hematopoietic and lymphopoid functions. From this period onwards, polypotent stem cells and lymphocyte precursors of hematopoietic tissue are formed only in the bone marrow.

Lymphocytes

Mature lymphocytes are divided into two populations, B- and T-lymphocytes.

B – lymphocytes

T – lymphocytes

O - lymphocytes

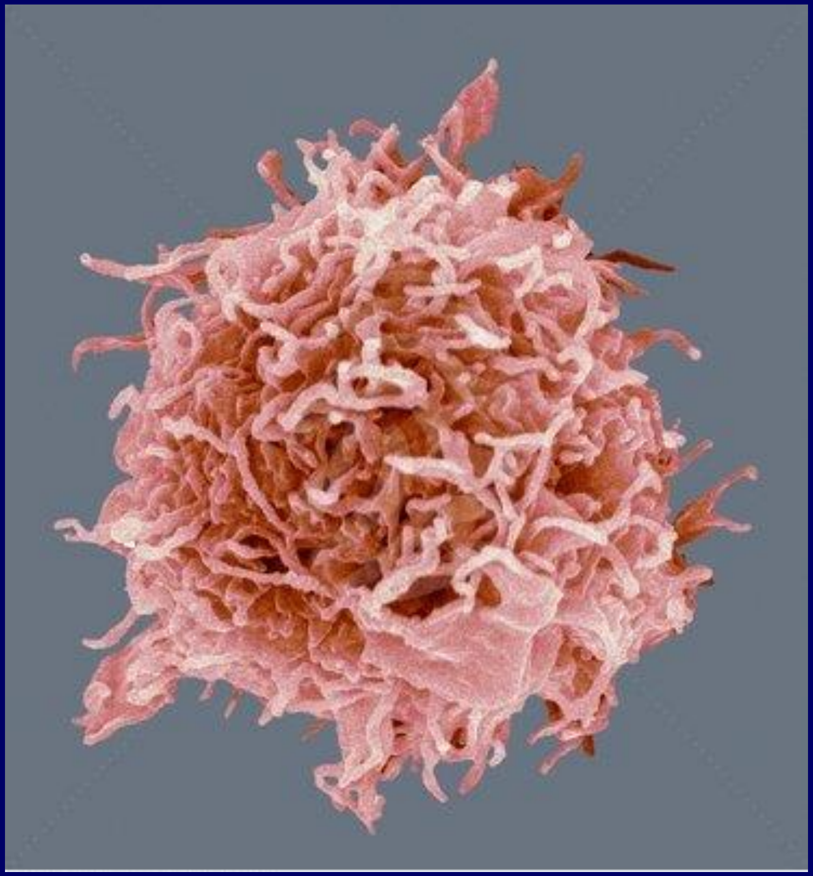
B-lymphocytes

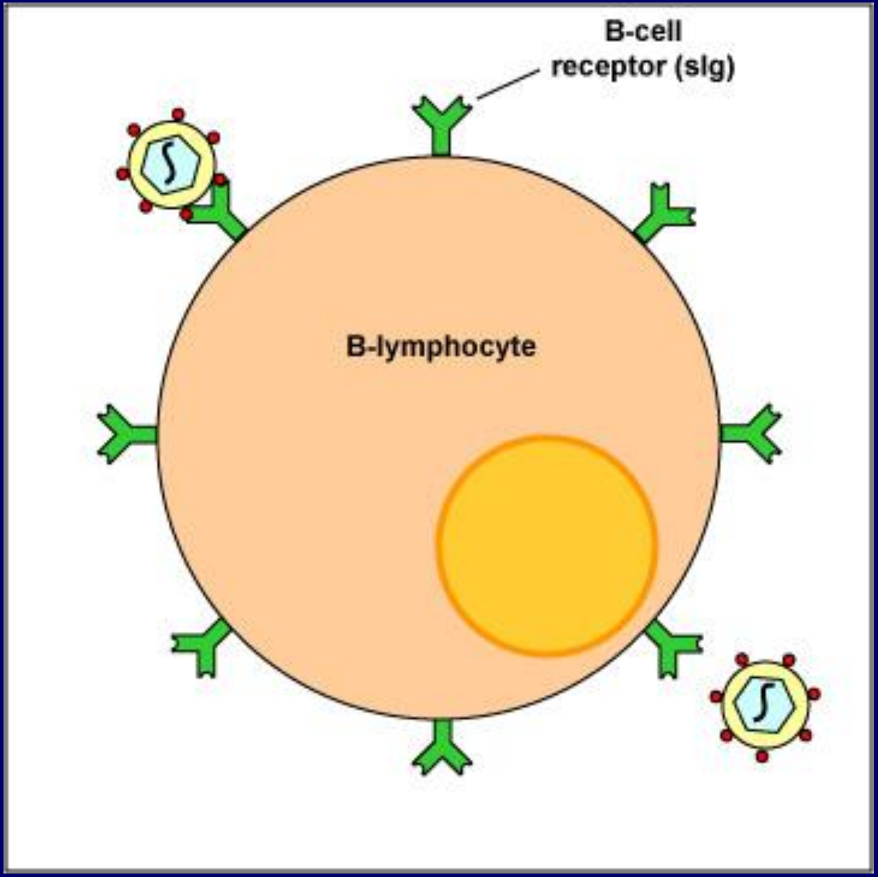
- B-lymphocytes are formed in birds in an organ called the Fabrisius sac, and in mammals in equivalent organs by differentiation from polypotent stem cells, regardless of antigen (hence the name B-lymphocytes, «bursa» - sac).
- In birds, the Fabrisius sac is a lymphoepithelial organ located in the posterior wall of the cloaca (secretory foramen).

B-lymphocytes and plasmocytes

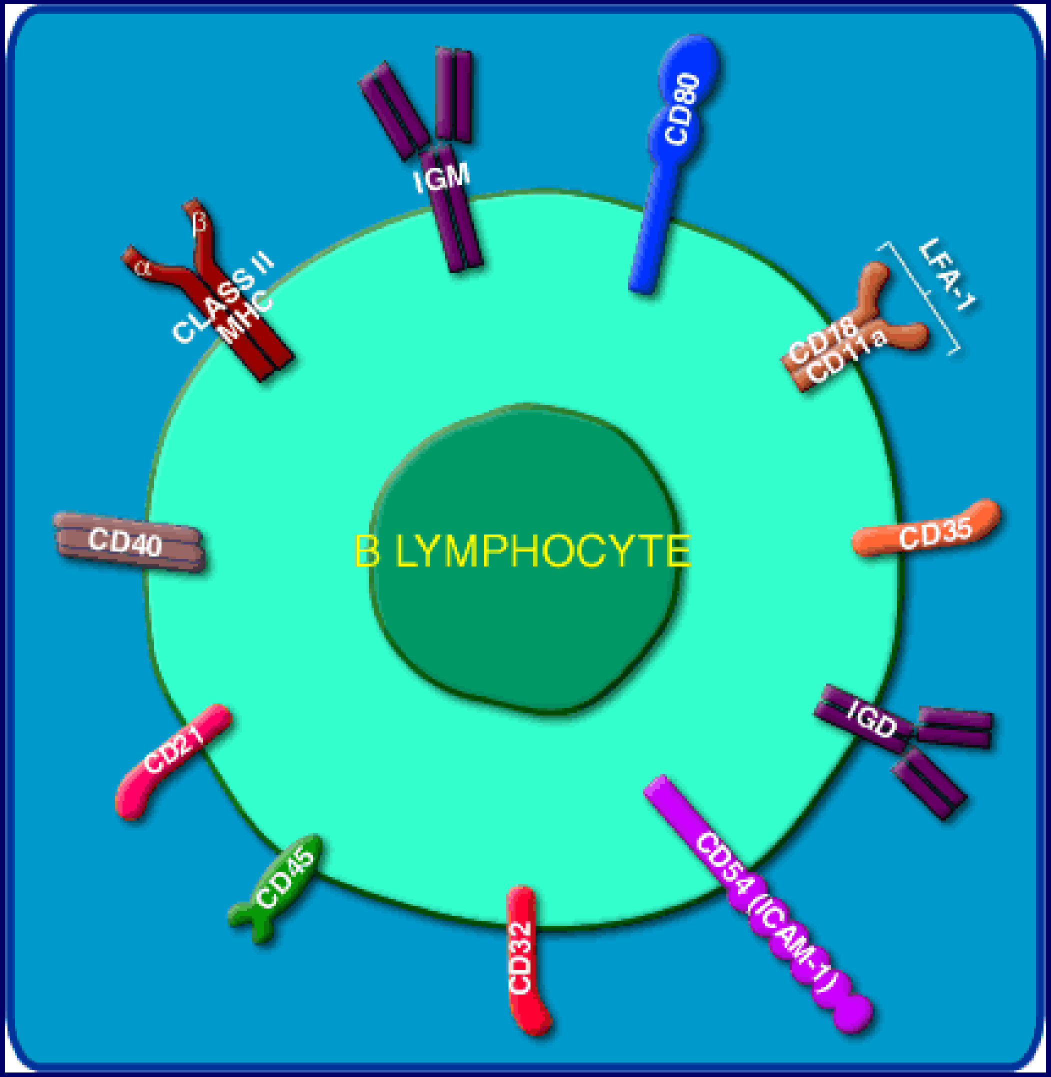
- **Provides humoral immunity by being antibody producers**
- **Participates in the formation of immunological memory**
- **Participates in immediate hypersensitivity reactions**

B-lymphocytes

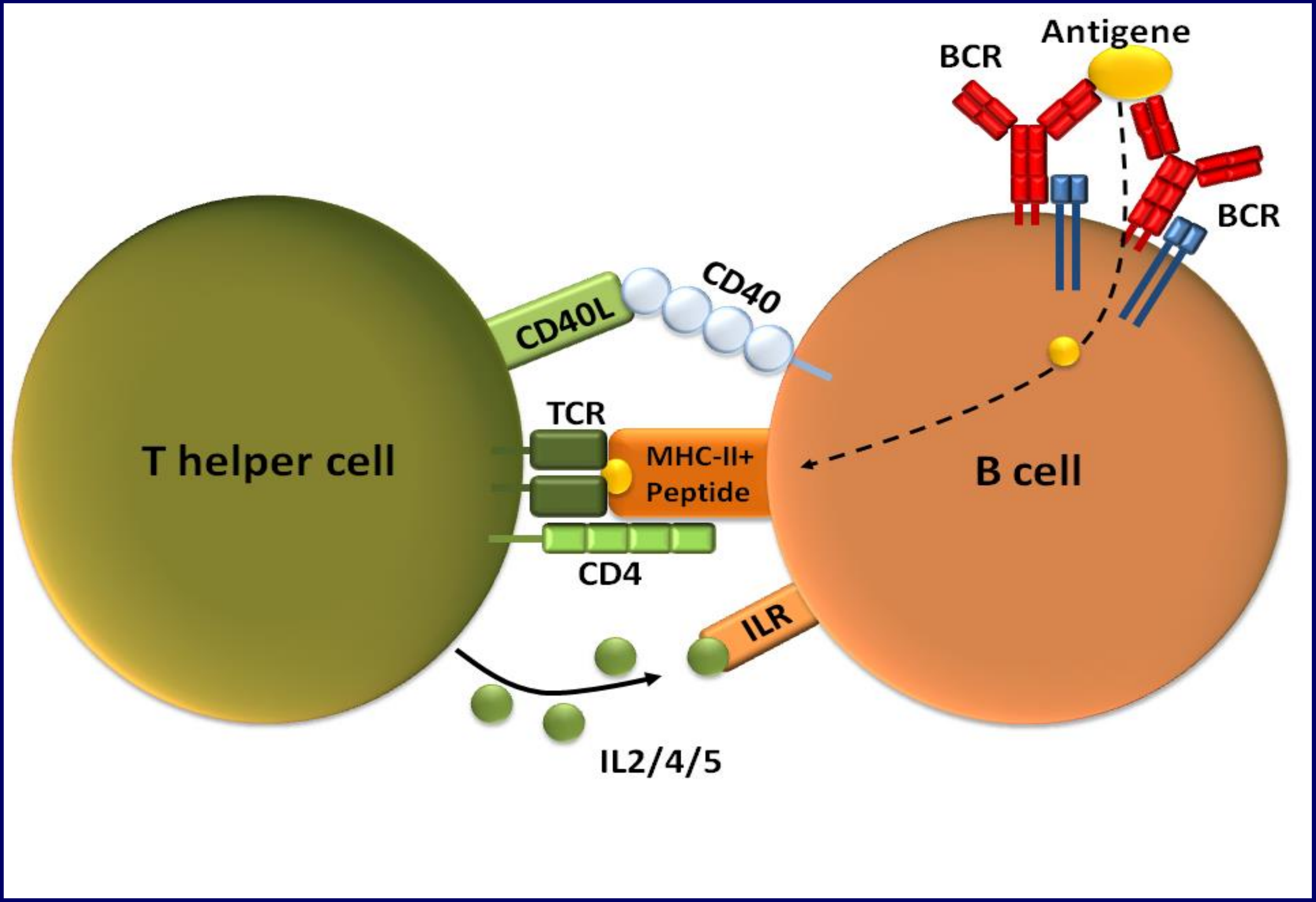




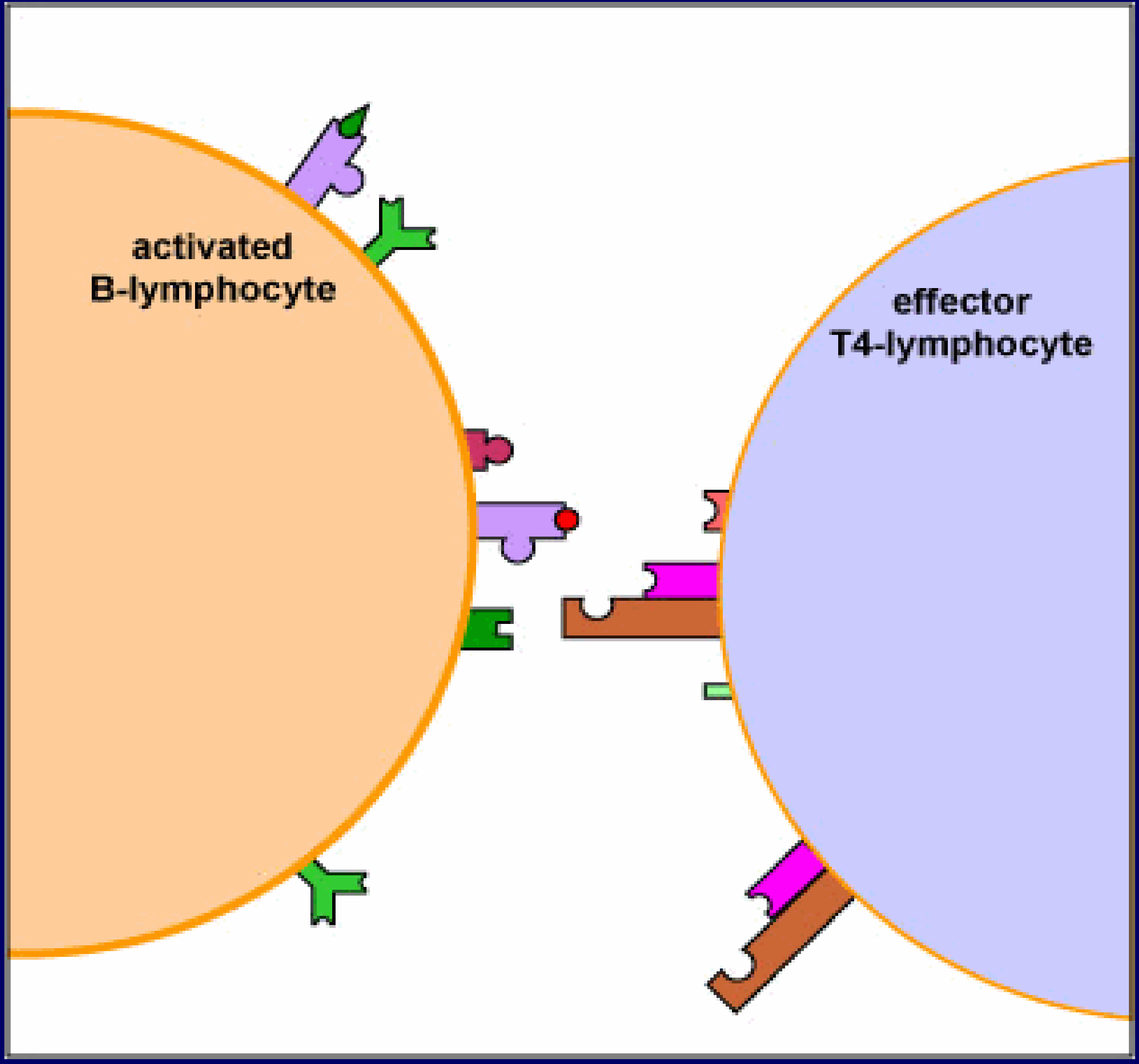
Receptors for B-lymphocytes



B-lymphocytes receive antigen information from T-helpers



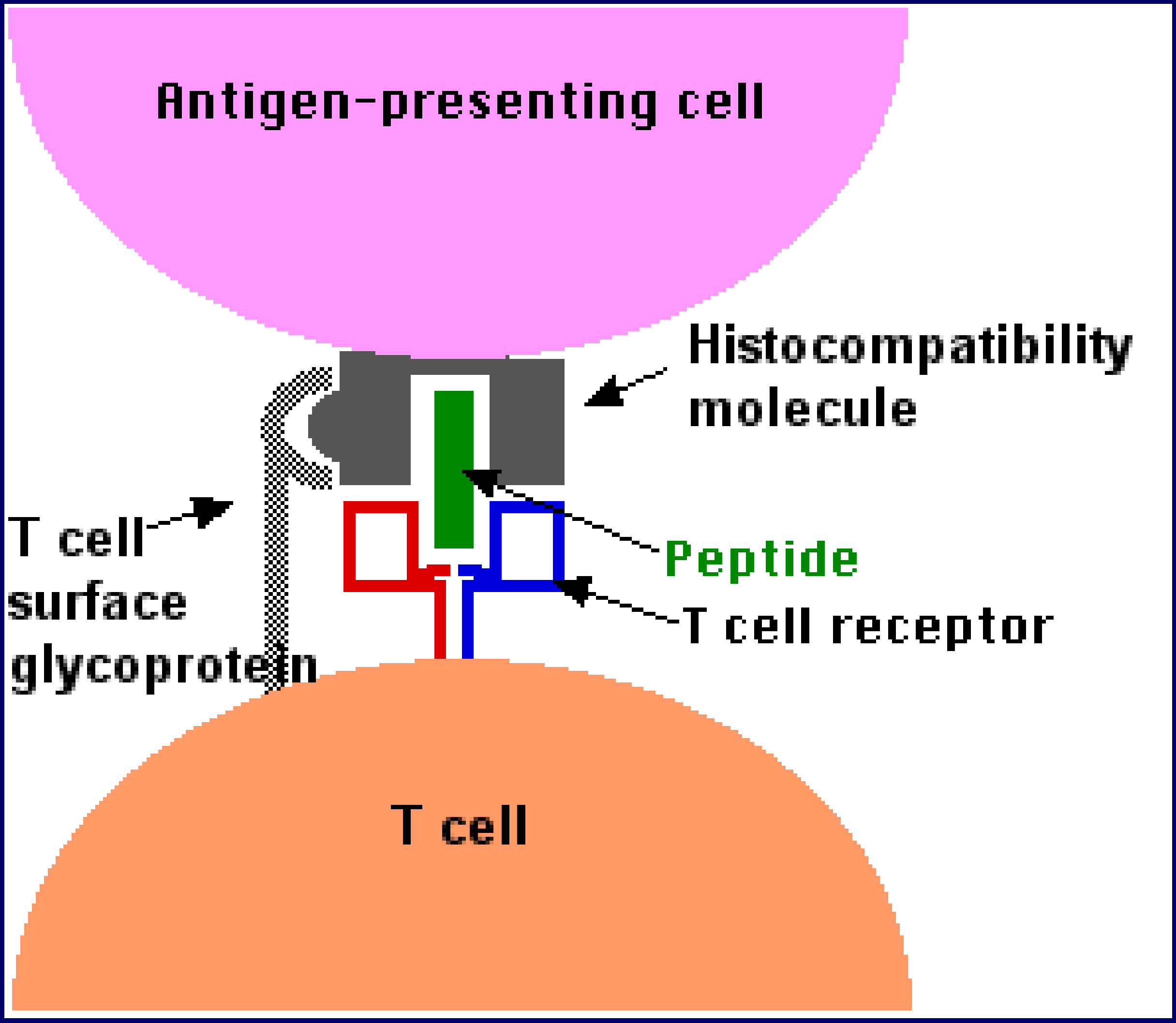
B-lymphocytes receive antigen information from T-helpers



T-lymphocytes

- **T-helpers (CD4) receives information from antigen-presenting cells and transmits it to other immunocompetent cells**
- **T-killers (CD8) destroys target cells with antibody-independent cytotoxicity**
- **T-suppressors perform an immunoregulatory function by weakening the immune response**

T-helpers receive antigen information from macrophages



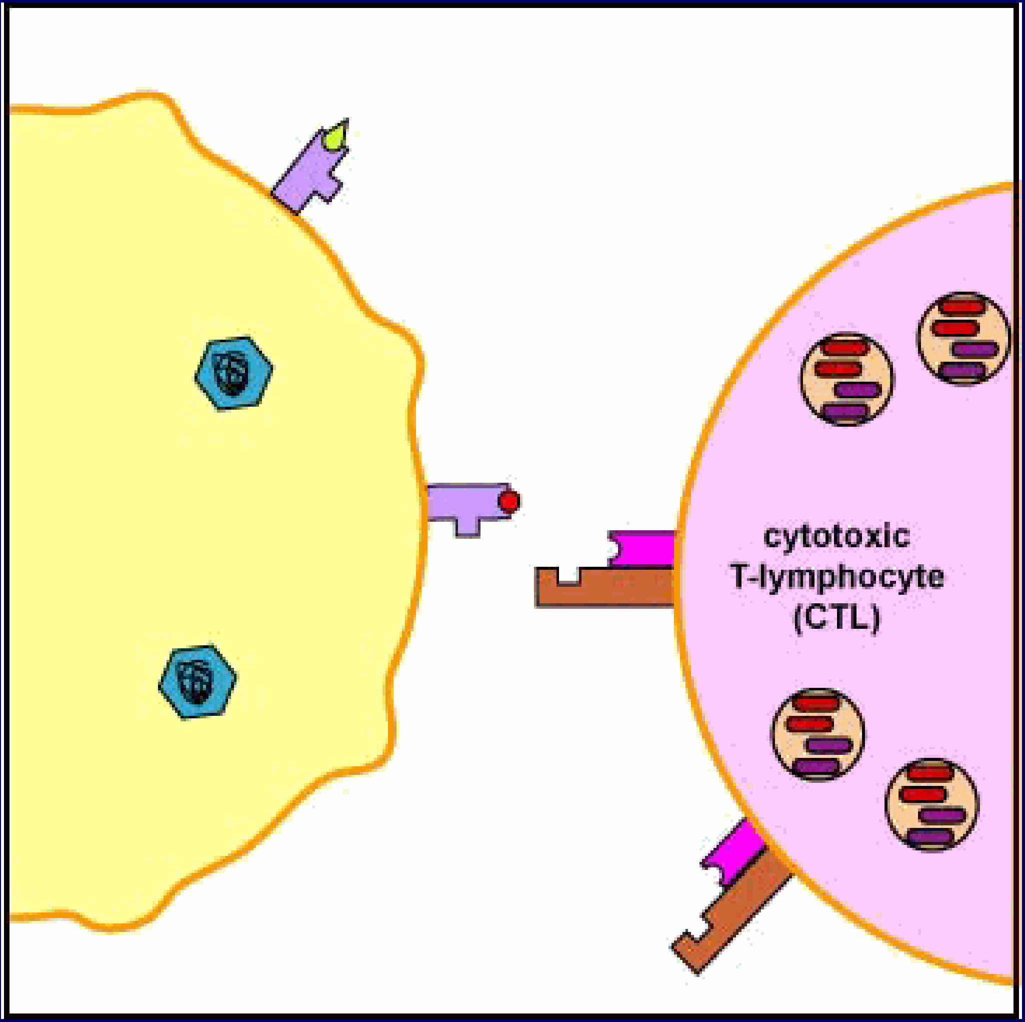
T-helpers (CD4)

- TH1- helper - provides a cellular immune response
- TH2- helper - provides a humoral immune response
- TH3-helper - maintains the balance between TH1 and TH2 by inhibiting the mechanism due to the production of beta-transforming growth factor (beta-TGF)

TH1/TH2 balance

- TH1- helper
- TH2- helper
- TH3- helper, or regulator T-helper

T-killers destroy target cells with antibody-independent cytotoxicity

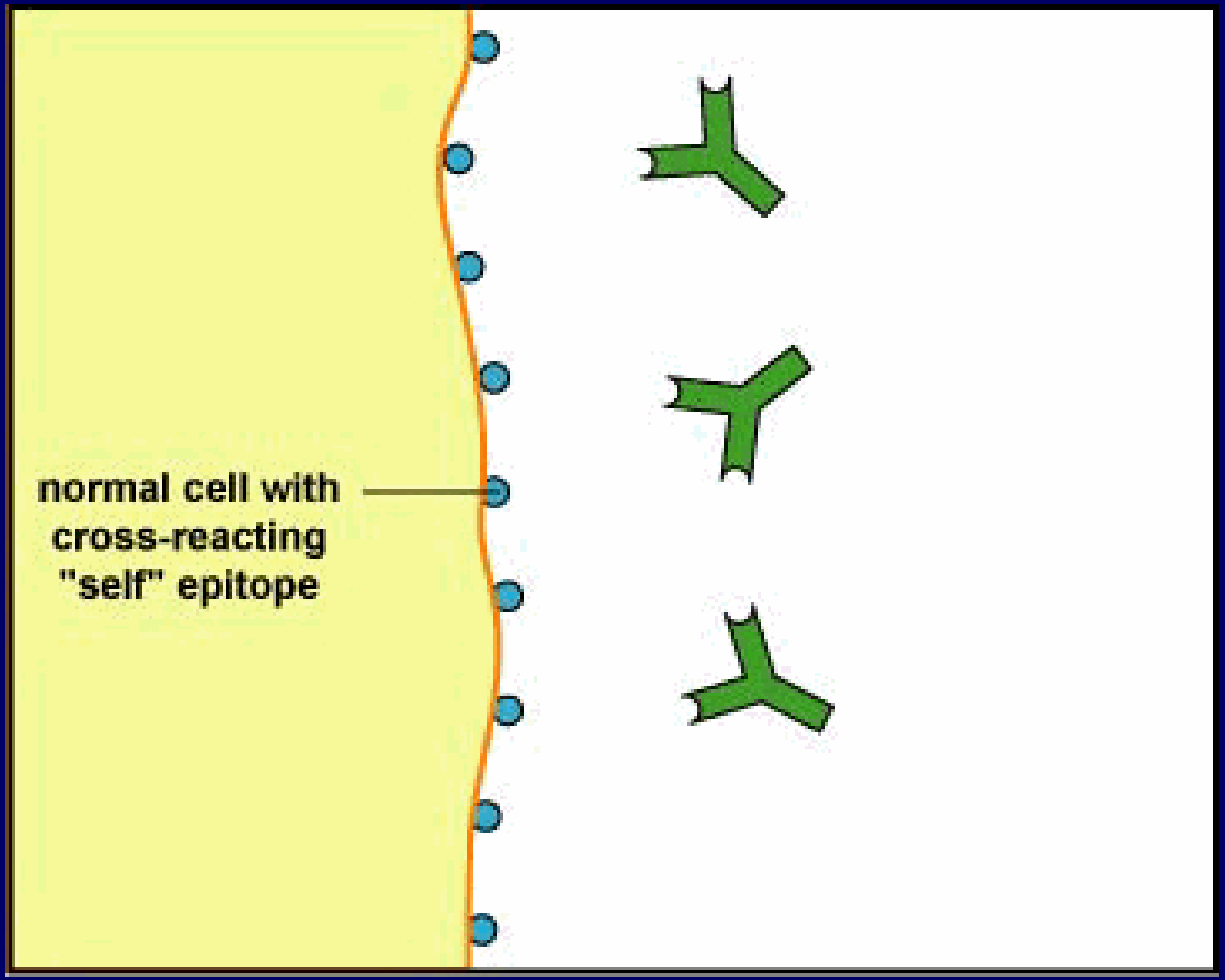


NK- cells
(eng. «natural killer»)

- Intracellular parasites and genetically modified cells (tumor cells) are the body's main defense cells
- They act without a specific immune response
- Destroy target cells with or without antibody-dependent cytotoxicity

"Attack" of NC cells on tumor cells

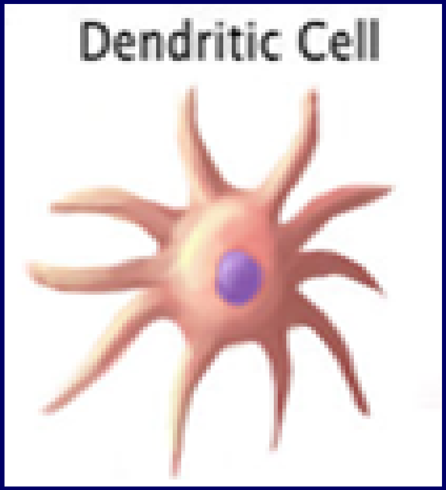
Effect of NC cells on the target cell



Other cells of the immune system

Dendritic cells

- Dendritic cells - have protrusions (the name of the cells is associated with it) in lymphoid and barrier tissues - especially in the epidermis of the skin (Langerhans cells), lymph nodes (interdigital cells), thymus
- Class II MHC is expressed on the surface of these cells. Being the most active antigen-presenting cells, it is possible to absorb, process (processing) the antigen by endocytosis and present it to the T-helpers in the complex with class II MHC (presentation).



Eosinophils

- Eosinophils are granular leukocytes, found in the blood and connective tissue, are the effector cells of IHS reactions.
- The helminths accumulate in large quantities in the site of local inflammation and perform the function of killers with antibody-dependent cellular cytotoxicity.
- Eosinophils "recognize" and activate parasites associated with these antibodies through receptors against IgA or IgE in the membrane.
- The activated cell secretes a number of toxic substances, which have a destructive effect on helminths.

Basophils

- Basophils can also be attributed to cells involved in nonspecific defense. They are also granular leukocytes, constantly circulating in the bloodstream.
- There are two types of basophilic cells in the mucous membranes and connective tissue.
- There are more of them in the skin, and under physiological conditions they perform an effector function and participate in immune responses in the skin-associated immune system.

Mast cells

- Myeloid`s cells, located in the barrier tissues - mucous membranes and subcutaneous connective tissue.
- According to the spectrum and localization of the biologically active compounds they synthesize, there are two types of barrier cells - mucous membrane and connective tissue cells.

Erythrocytes and platelets

- **Erythrocytes participate in the immune defense by producing erythropoietin, which not only stimulates hematopoiesis, but also provides immune support to erythrocytes, as well as other blood cells, including the immune system.**
- **Platelets, which produce the majority of serotonin, can also be classified as defense cells, given their participation in the defense against cancer.**

Specific immunity

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

Antigens

- **Antigens are genetic substances that stimulate this or that specific immune response in the body (synthesis of antibodies, cellular immune reactions).**
- **Antigens can be both chemically pure substances purified from mixtures (eg, serum albumin, egg albumin, purified microbial toxin, etc.), as well as complex drugs, cells, and tissues.**

Antigens

- **Antigens are primarily protein-containing substances.**
- **However, the antigenic property is not limited to proteins, but also to many complex polysaccharides, lipopolysaccharides, polypeptides, as well as some artificial polymeric compounds, ie organisms in the body.**

Features of antigens:

- Foreignness - is an inseparable feature of antigen. Antigens must first be foreign to the appropriate organism.
- However, even antigenic determinants of non-genetically related animals or biopolymers of different structures may have certain similarities. These are called cross-antigens.
- The antigens of some microorganisms cannot be recognized by immune factors because they are similar to the antigens of the human body, a phenomenon known as antigenic mimicry.

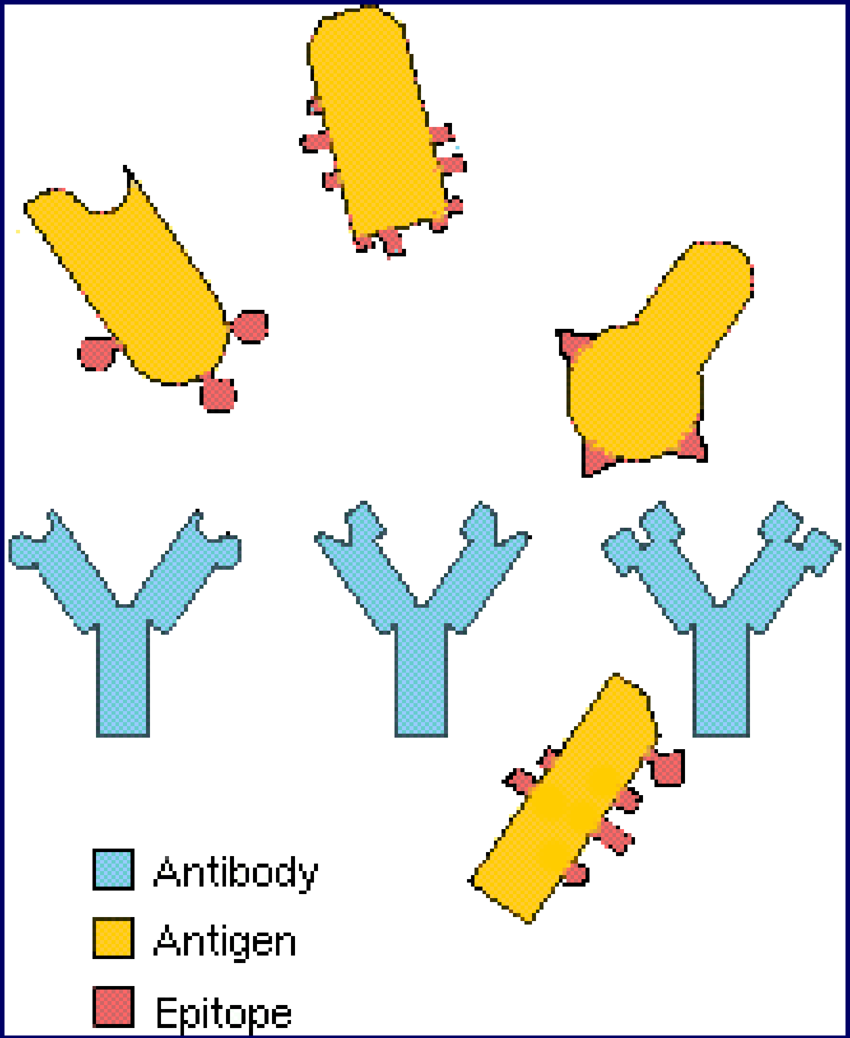
Features of antigens:

- Xeno-, allo- and isoantigens are distinguished according to the degree of memory.
- Xenogens, or heterologous antigens, are the same for organisms belonging to different genera and species.
- Allogen or group antigens are common to genetically different organisms of the same species. Based on alloantigens, the general populations of organisms can be divided into separate groups. For example, blood group antigens in humans are an example of such antigens.
- Isogenic or individual antigens are common only to genetic identical organisms, such as identical twins, inbreeding animals, and genetic clones.

Features of antigens:

- **Antigenicity - characterizes the ability of antigen to induce antibodies.**
- **Not all antigen molecules have antigenic properties. This molecule contains antigenic determinants, or small chemical structures called epitopes, that provide its antigenicity. They induce the synthesis of antibodies and bind to them.**
- **Each antigen may have one or more determinants. Most antigens have many determinants, ie they are multivalent.**

Antigenic determinants, or epitopes



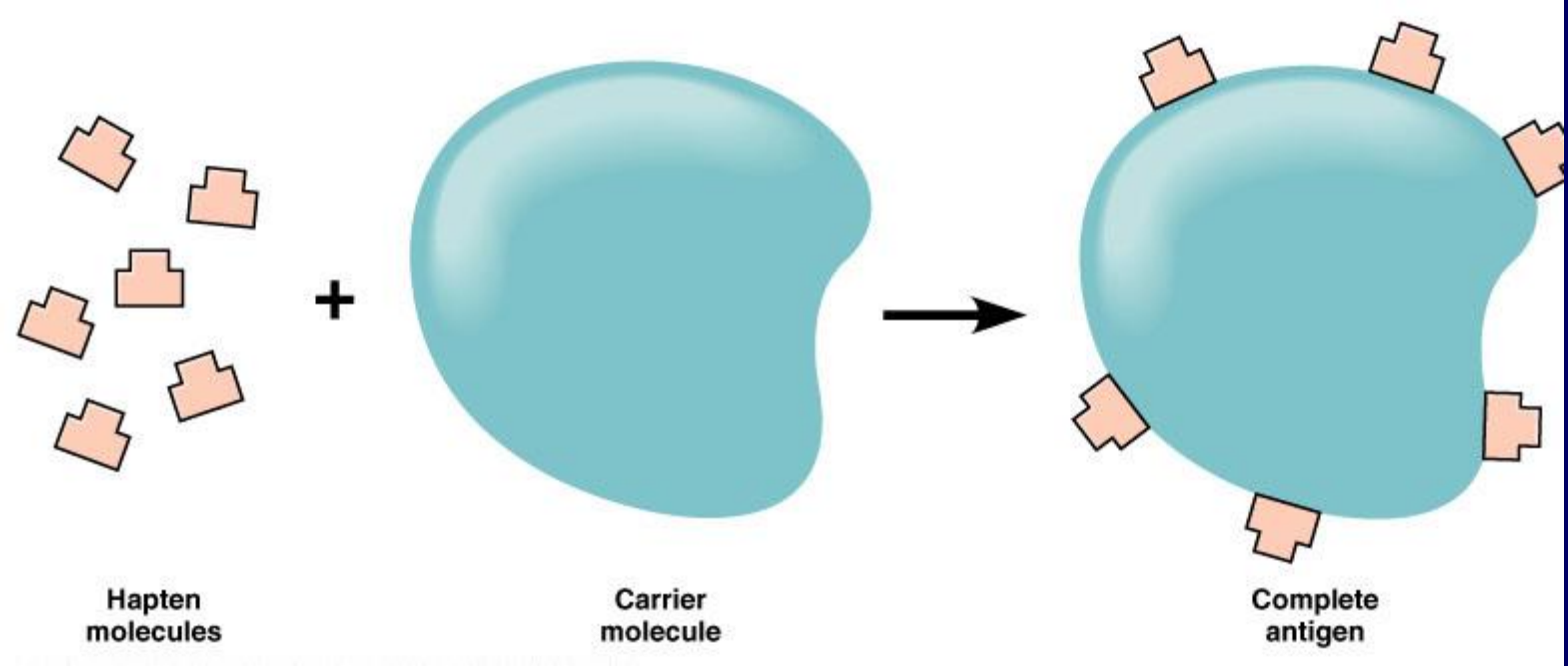
Features of antigens:

- Immunogenicity is the ability of an antigen to form (form) immunity.
- The degree of immunogenicity depends on a number of factors - the molecular structure of the antigen and the reactivity of the macroorganism.
- Despite their similarities, there are differences between the concepts of antigenicity and immunogenicity. For example, the causative agents of bacterial dysentery have a high antigenicity, but they do not form a sufficient immunity against the disease, in other words, the immunogenicity of these pathogens is weak.

Haptens

- ***Haptens, or half-antigens, although they have enough antigen, do not have immunogenicity.***
- ***Haptens are small-molecule, non-protein substances,***
- ***Once they combine with so-called "carrier" proteins, they acquire antigenic properties.***

Haptens



Features of antigens:

- Specificity is the ability of antigens to induce a specific immune response in the body.
- The interaction of antigens and antibodies is highly specific. This feature is widely used in diagnostic laboratories for the identification of microorganisms.
- The binding force of an antigen to an antibody - affinity - can change in proportion to the similarity of their areas of association. Antigens differ in their affinity.

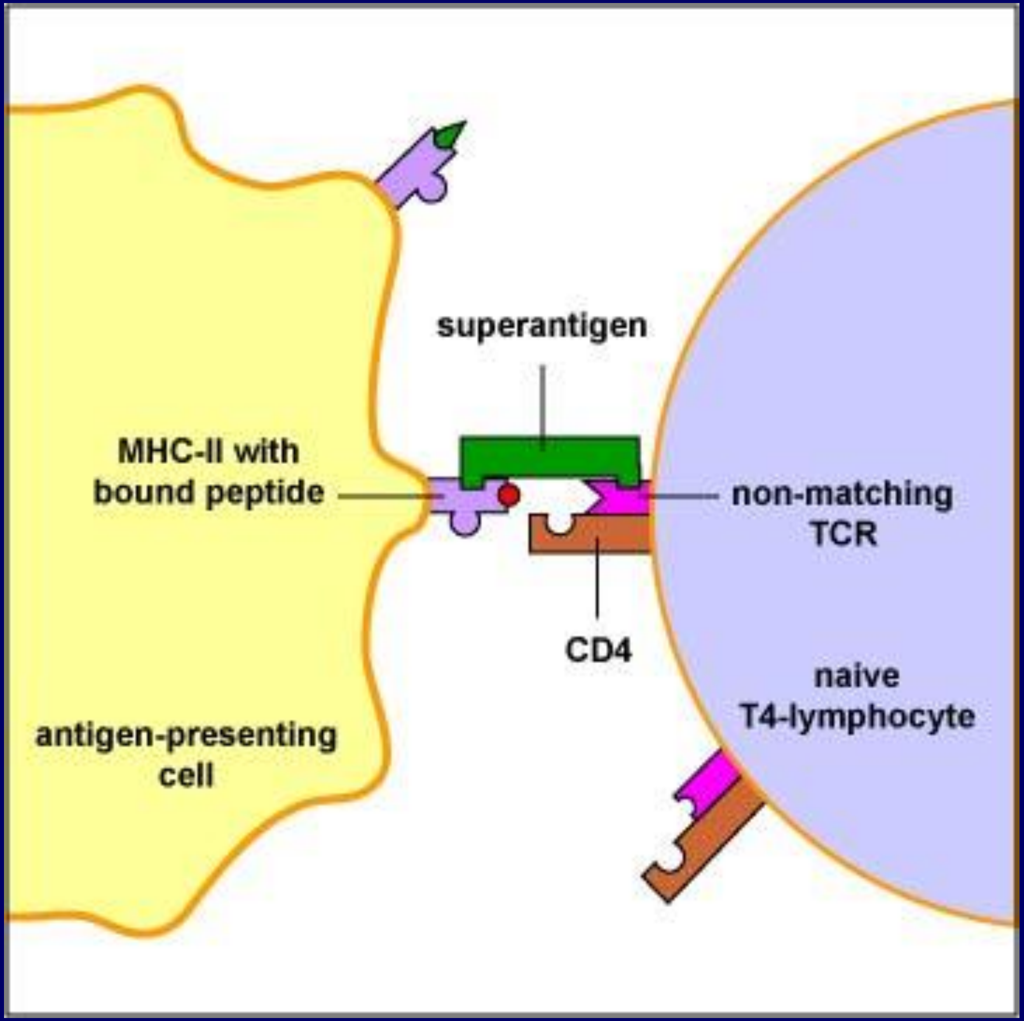
Immunogens, tolerogens and allergens

- When immunogens enter the body, they are able to induce productive immune responses resulting from the production of immune factors (antibodies, antigen-reactive lymphocyte clones).- T-dependent and non-T-dependent antigens
- Tolerogens - in contrast to immunogens, cause tolerance or reactivity in the body. Tolerogen molecules are characterized by high dispersion due to their monomerism, small molecular weight, high density of epitopes.
- Allergens do not differ from immunogens in their properties, they cause the formation of sudden or slow hypersensitivity reactions in the body.

Superantigens

- **Some antigens of microbial origin can activate T-helpers by penetrating APC and T-helper cooperation without undergoing processing.**
- **These antigens, called superantigens, bind freely to the "class II MHC antigen - T-cell receptor" complex and form a false signal.**

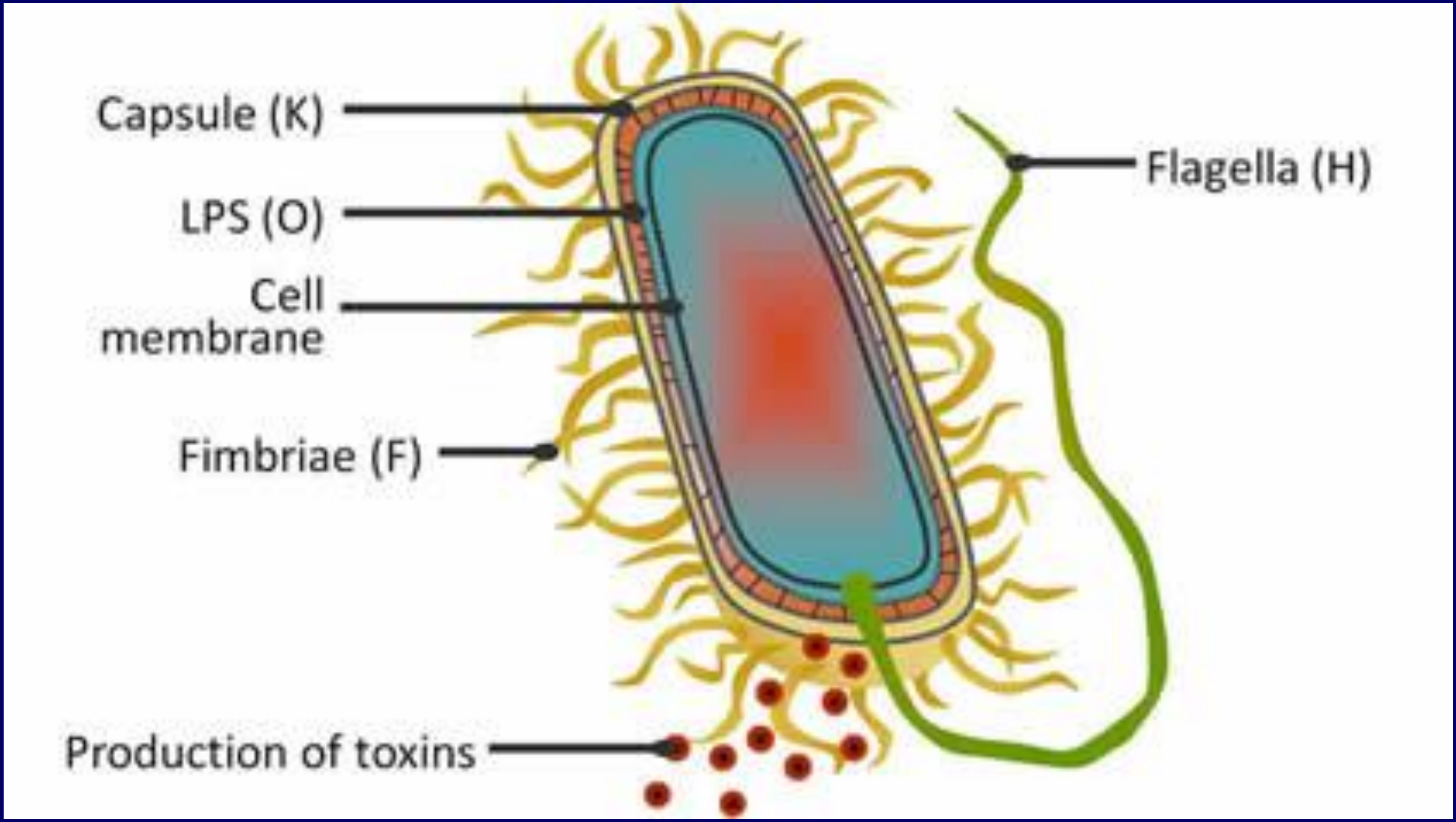
Superantigens



Antigens of microorganisms

- Bacterial antigens
- Flagella antigen, or H-antigen
- Somatic, or O-antigen
- Capsule, or K-antigen
- virulence antigen, or Vi-antigen
- Exotoxins, enzymes
- Virus antigens
- virus-specific antigens

Bacterial antigens



Antigens of the human body

- ***Erythrocyte antigens***
- ***ABO system antigens***
- ***rhesus antigens***
- ***Main Complex of Tissue Compatibility (English, Main Hystocompatibility Complex - MHC, Human Leukocyte Antigen - HLA)***
- ***There are two main classes of the MHC molecule.***
- ***Class I MHC in all nucleated cells,***
- ***Class II MHC is mainly expressed on the surface of immunocompetent cells.***

Tissue compatibility antigens

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

MHC

- **MHC in humans is called HLA (Human Leukocyte Antigen) because it was first detected in leukocytes.**
- **The biosynthesis of HLA 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).**
- **Locations III and (sometimes called Class III) are located between Class I and Class II loci. At this locus are the genes that encode the two components of the complement (C2 and C4).**

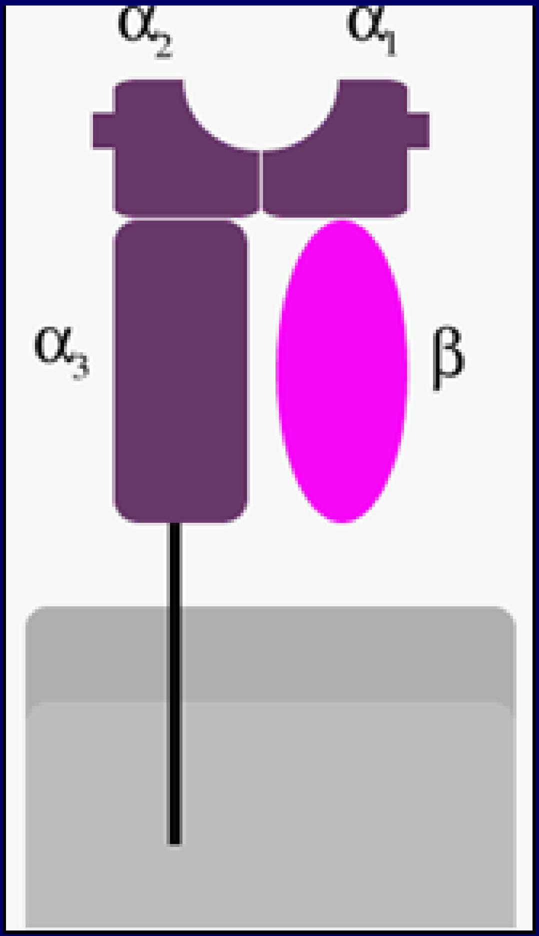
MHC

- **Thus, there are two main classes of the MHC molecule.**
- **Class I MHC in all nucleated cells,**
- **Class II MHC is mainly expressed on the surface of immunocompetent cells.**
- **There are no individuals in the entire human population who have the same MHC antigens, in other words, all people differ in these antigens. But the exception is single-egg twins, as well as genetic clones. Therefore, the compatibility of these antigens, or more precisely, their relative compatibility, is taken into account in tissue transplants.**

MHC structural features and functions

- Due to their chemical nature, MHC antigens are glycoproteins that bind tightly to the cytoplasmic membrane of cells.
- Separate fragments have a homologous structure with immunoglobulin molecules.

Class I MHC proteins are glycoproteins and are found on the surface of virtually all nuclear cells.



Class I MHC provides the biological individuality of each organism, as if it were a biological passport and a "native" marker for the immunocompetent cells of that organism.

- **Cell infection and mutations change the structure of class I MHC.**
- **The Class I MHC molecule, which contains foreign or modified peptides, activates T-killers (CD8 + lymphocytes) due to the fact that it has a structure that is not characteristic of the organism.**
- **Thus, cells that differ in class I MHC antigens are destroyed as foreign cells.**

I class MHC

- According to the first class MHC, the definition of the type of individuals is important in transplantology.
- It is determined by serological methods - by cytolytic reactions of lymphocytes with specific serums.
- This reaction is carried out with the help of monoclonal antibodies against class I MHC antigens.

II class MHC

Class II MHC proteins are also glycoproteins, which are found in the surface of certain cells in the body, including macrophages, T-helpers, B-lymphocytes, dendritic cells of the spleen, and Langerhans in the skin.

Class II MHC differs from Class I MHC in structure and functions due to the following features.

- **Class II MHC is expressed not on the surface of all cells, but on the surface of some cells, especially immunocompetent cells.**
- **Class II MHCs include peptides that are not synthesized in the cell itself, but are captured by endocytosis from the extracellular environment, such as intracellular viral antigens.**

II class MHC

- **The synthesis of class II MHC takes place in the endoplasmic reticulum, the formed dimer complex then enters the cytoplasmic membrane of the cell.**
- **Class II MHC antigen is expressed on the cell surface within 1 hour after endositosis.**

Class II MHC participates in the induction of the immune response.

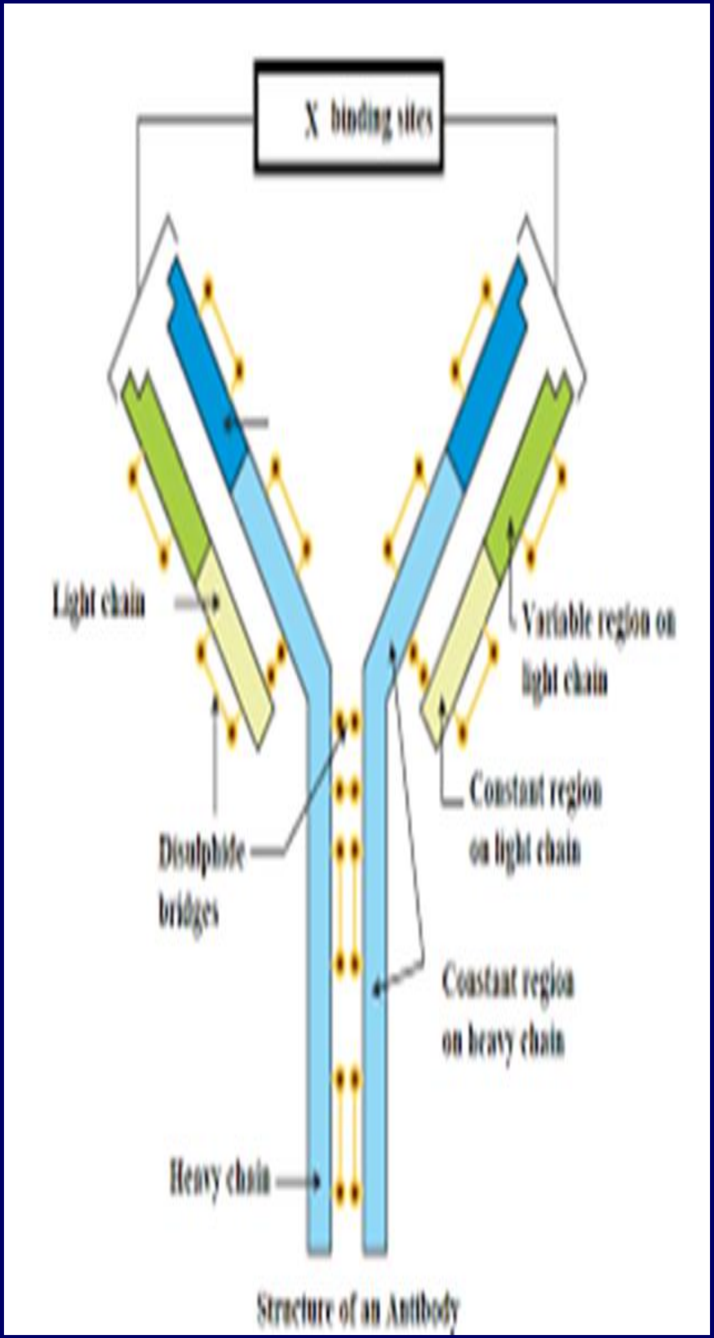
- **This process takes place as follows:**
- **Fragments of the antigen molecule are expressed on the surface of the antigen-presenting cell (dendritic cell, macrophage, etc.) in the form of a complex "class II MHC + antigen".**
- **This complex is recognized and analyzed by T-helpers (CD4 + lymphocytes).When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

CD-antigens

- In the membrane of the cell there are a group of antigens - markers that unite them according to similar morpho-functional properties. Among them, the markers of immunocompetent cells have been studied in more detail.
- These marker molecules are called cell differentiating antigens, or CD antigens (cell differential antigen). These are glycoproteins in structure and some are immunoglobulin in nature.

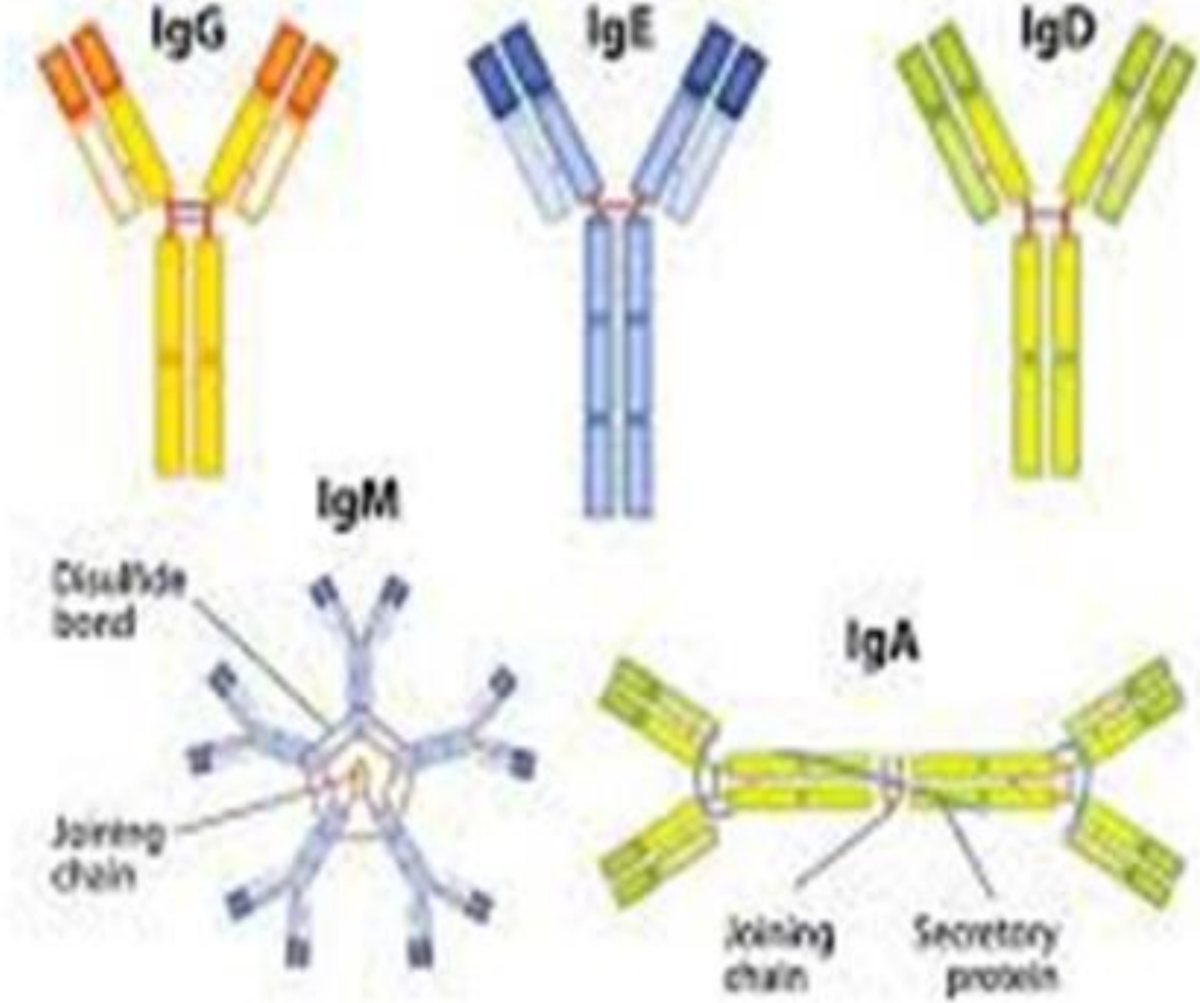
Immunoglobulins or antibodies

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



- 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). 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 withnoncovalent electrostatic, van-der-vaals, hydrogen and hydrophobe bonds. H and L chains have constant domens calledFc- fragments (fragment crystallisable) with different function. This fragment is ableto 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.
- Depending on antigenic features 5 classes of Hchain exist - a, m, g , e, d. Accordingly, 5 classes of immunoglobulins are distinguished. Antibody with a-type chain is called IgA, m-chain- IgM, g-chain -IgG, e-IgE, d-chain- IgD. Some classes of Ig have subclasses: IgG - 4 (IgG1, IgG2, IgG3, IgG4), IgA, IgM and IgD classes have 2 subclasses.

Immunoglobulins



ANTIBODIES

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. 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. 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)

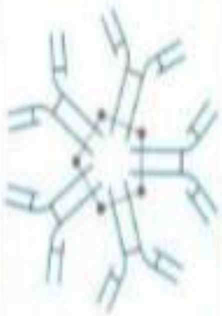
It makes up 10-15% of immunoglobulins of blood serum. Has two subclasses - IgA1 and IgA2. IgA1 is present in the serum, while IgA2 is a part of sIgA and predominant in the secretions. IgA2 is resistant to the action of proteolytic enzymes of saliva, secretions of intestinal mucosa. The secretory component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

E immunoglobulin (IgE) :

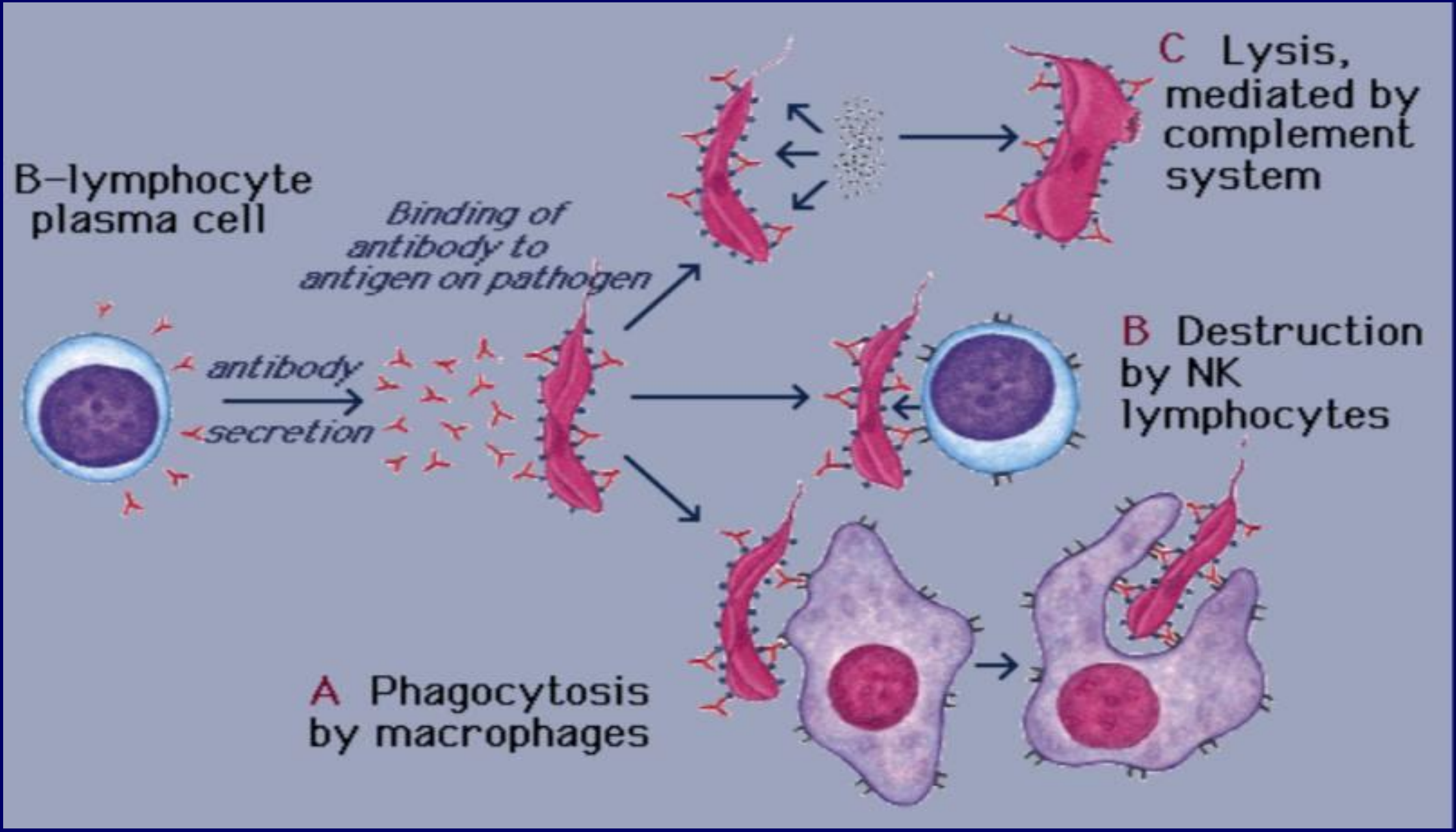
It differs from other immunoglobulins by its high cytophilicity - ability to bind to mast cells and basophils. This immunoglobulin has two important biological features:1) it provides immediate type hypersensitivity reactions;2) it is involved in the body's defense response during parasitic diseases,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

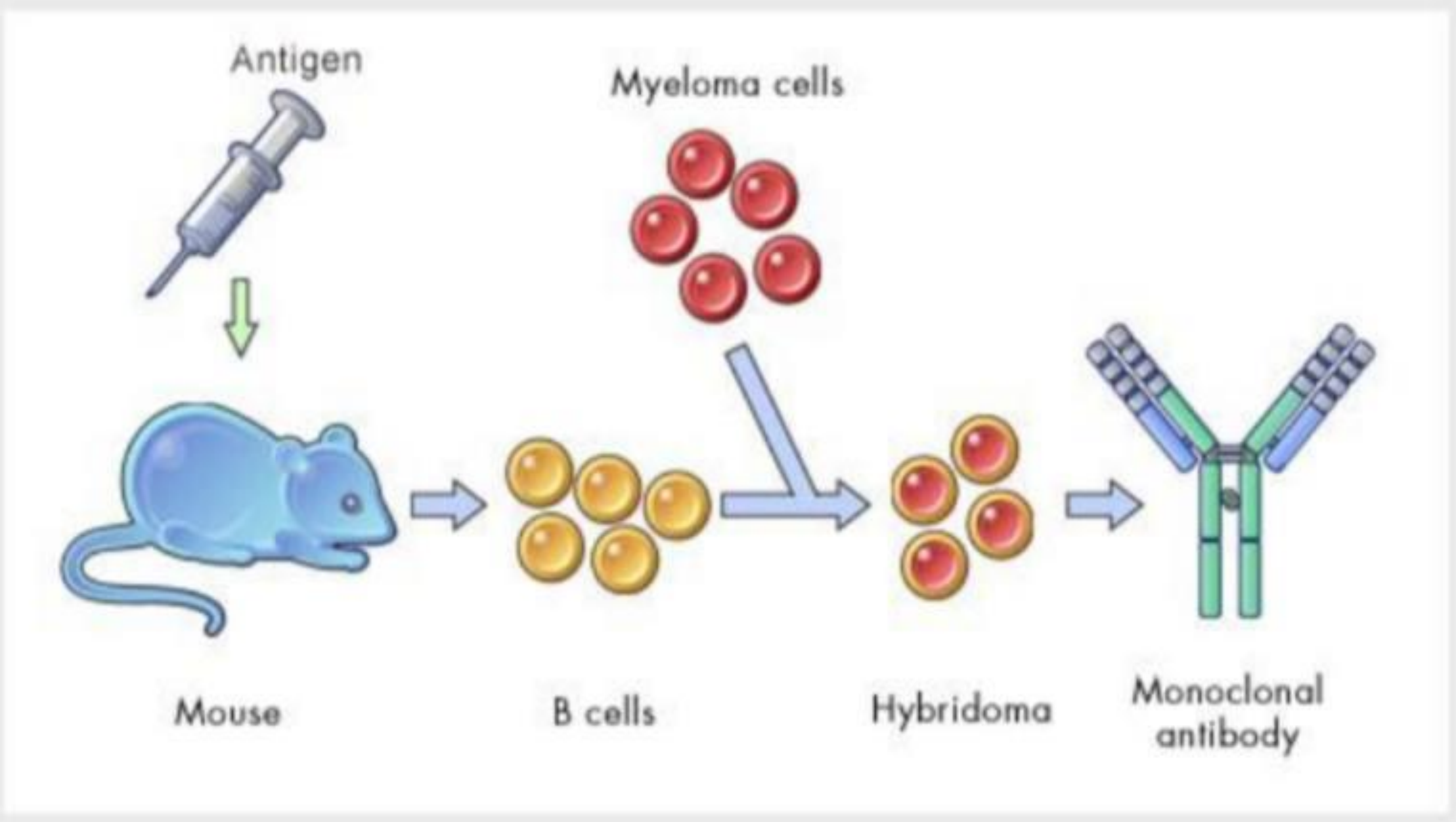
The antibody function of this immunoglobulin is unknown, but it acts as an antigen-receptor on the surface of the precursors of B-lymphocytes. IgD is present in blood serum in small amounts - 0.03 g / l (0.2% of all circulating immunoglobulins). It has a molecular weight of 160,000 D and is a monomer.

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

Antibodies function



Obtaining monoclonal antibodies



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 antigenbinding centers.