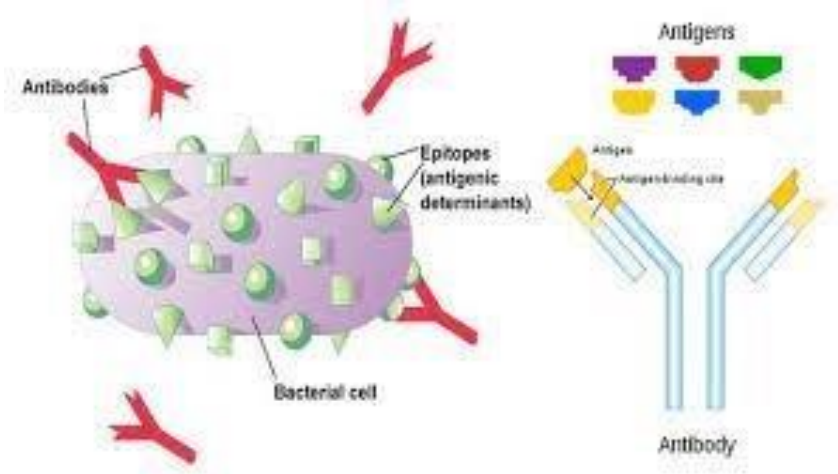
Practical 9
Acquired (specific) immunity, its types. Antigens, their types. Antigenic structure of microbial cell. The concept of the immune system. Immune competent cells. Immune response reactions. Antibodies. Serological tests

Specific immunity : Depends on type of antigen entering organism with help of specialfactors. 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 are commonly proteins. However, complex polysaccharides, lipopolysaccharides, polypeptides, some artificial polymeric compounds have antigenic properties aswell.



***Properties of antigen*:**

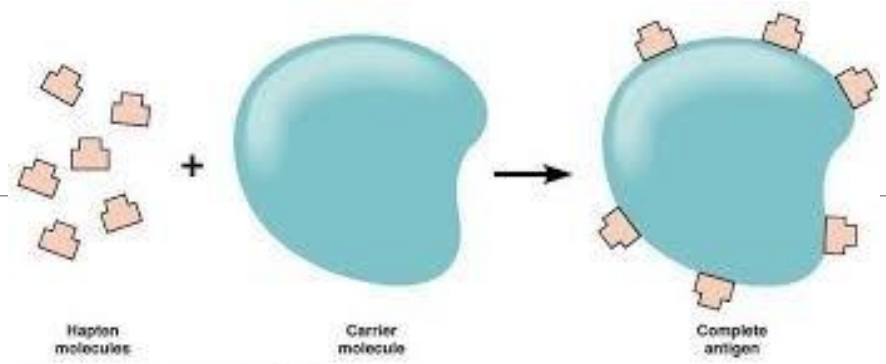
Foreigness– the main feature of antigen. An antigen must be a foreign substance for organism. However, antigenic determinants of genetically nonrelated animals or biopolymers may have similarities. They are called cross antigens. The antigens of some microorganisms are not recognized by immune factors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

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.

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.

Immunogenicity – ability of antigen to form immunity. Immunogenicity depends on molecular structure of antigen and reactivity ofmacroorganism. 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, or inncomplete antigens have antigenicity and weakimmunogenicity. They are small nonproteinic molecules that elicit an immune response only when attached to a large carrier such as a protein.



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 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.

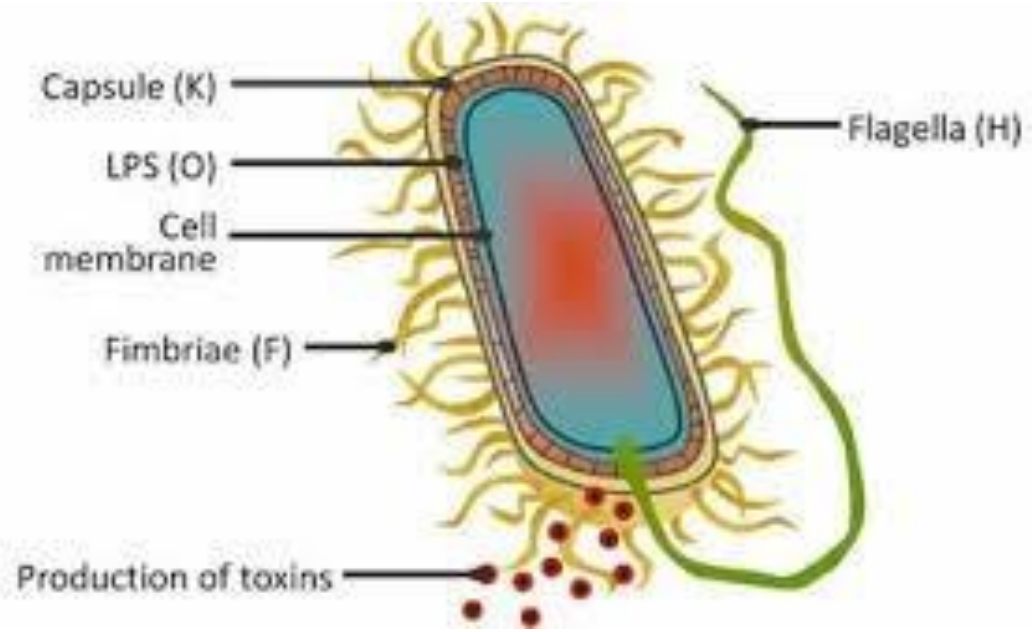
Some antigens can activate T-helpers without APC and Thelper cooperation. These molecules called **superantigens** can bind to MHC I I -TCR complex and form false signal.

Microorganism antigens :

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 : 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



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 allcells in the body. Most of them belong to the Main Hystocompatibility Complex (MHC) antigens.

MHC

Human MHC abtigen is called HLA as it first was described in leucocytes (Human Leuĸocyte 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 IIIis located between I and IIloci. The genes that encode the two components of the complement (C2 and C4) are located in this locus. 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 antigens are glycoproteins located on cell membrane. Some MHC fragments have homologous with immunoglobulins structure.

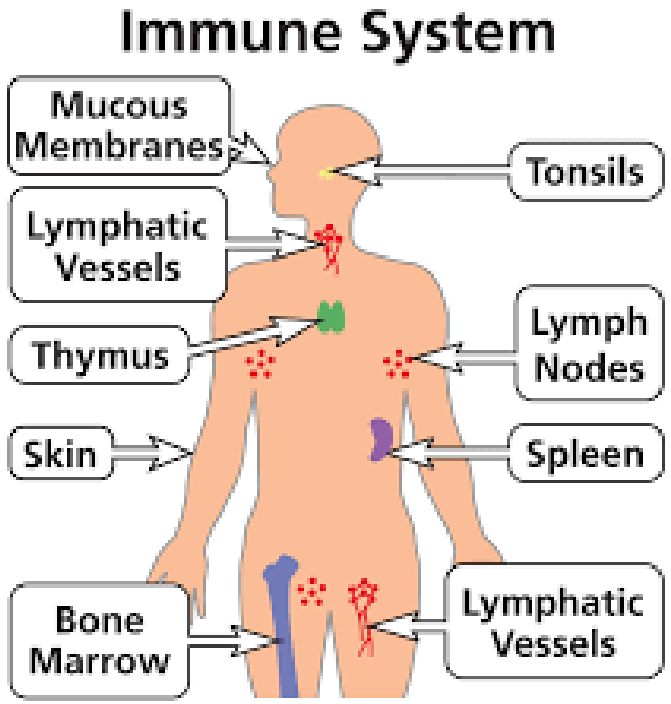
CD-antigens : In the membrane of the cell there are a group of antigens - markers that unite them according to similar morpho-functional properties. Among them, the markers of immunocompetent cells have been studied in more detail. These marker molecules are called cell differentiating antigens, or CD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

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.

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



Lymphocytes:

Mature lymphocytes have two subpopulations: • B - lymphocytes • T – lymphocytes • O – 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 Blymphocytes, «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: Create humoral immunity by synthesis of antibodies • Participate in development of immunological memory • Participate in immediate type immune responses

• 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

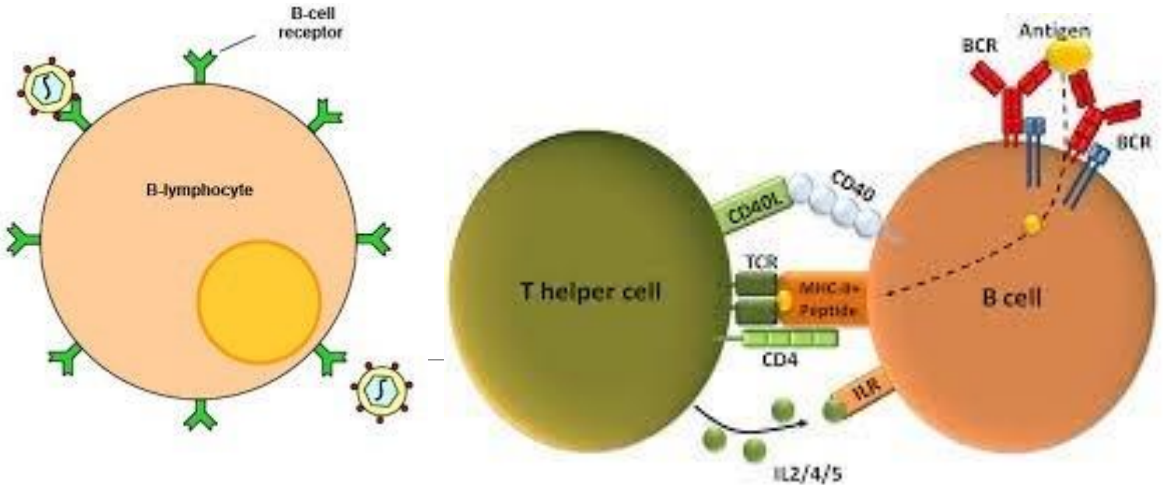
• T-suppressors perform an immunoregulatory function by weakening the immune response

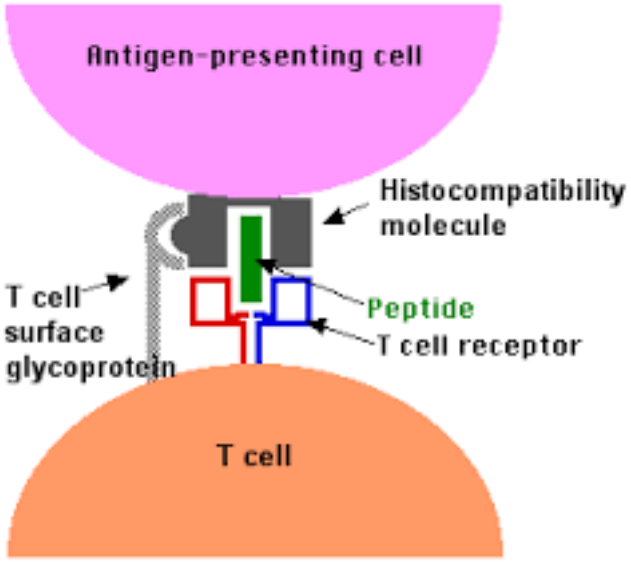
T-helpers receive antigen information from macrophages

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)





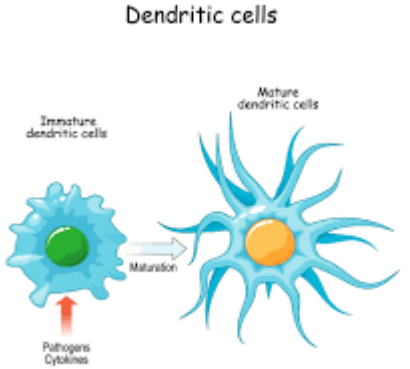
NK-cells (eng. «natural killer»)

• The main defensive cells against intracellular parasites andgenetically foreign cells (tumour cells)

• Act independently from specificimmunity

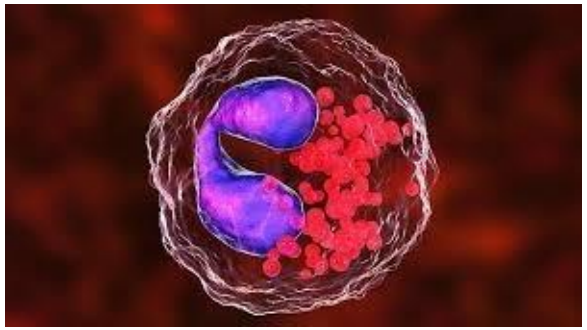
• Destroy target cells by antibody dependent and independent cell cytotoxicity

Other cells of the immune system:

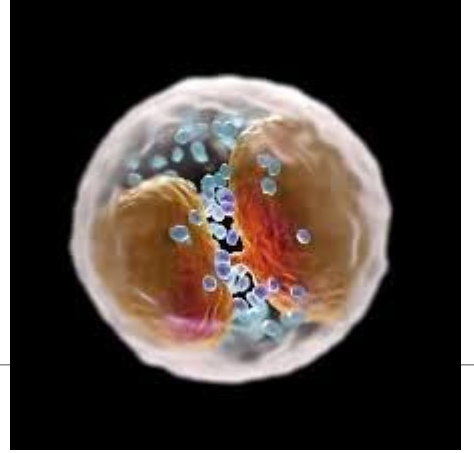


Dendritic cells - have protrusions (the name of the cells is associated with it) in lymphoid and barrier tissues - especially in the epidermis of the skin (Langerhans cells), lymph nodes (interdigital cells), thymus .Class II MHC is expressed on the surface of these cells. Being the most active antigen-presenting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

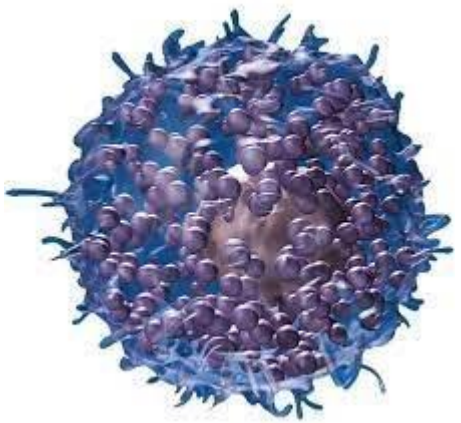
Eosinophils are granular leukocytes, found in the blood and connective tissue, are the effector cells of IHS reactions. The helminths accumulate in large quantities in the site of local inflammation and perform the function of killers with antibody-dependent cellular cytotoxicity. Eosinophils "recognize" 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.



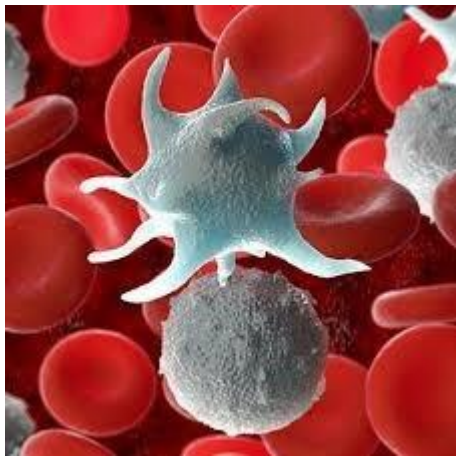
Basophils can also be attributed to cells involved in nonspecific defense. They are also granular leukocytes, constantly circulating in the bloodstream. There are two types of basophilic cells in the mucous membranes and connective tissue. There are more of them in the skin, and under physiological conditions 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.



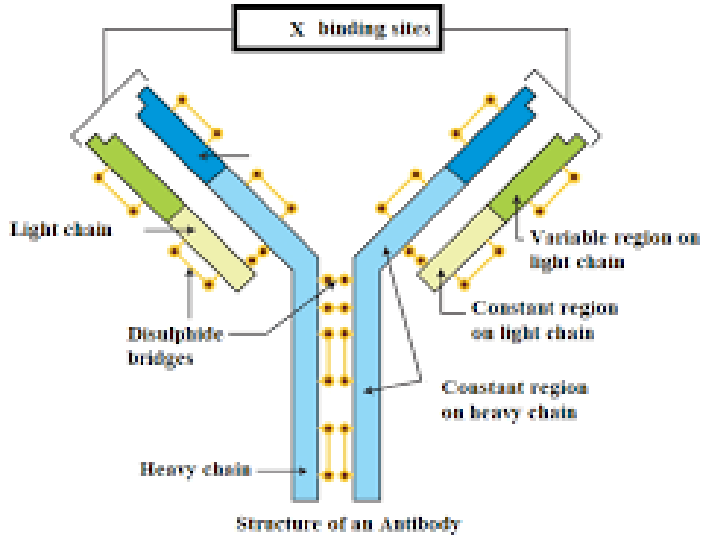
*Erythrocyte*s participate in the immune defense by producing erythropoietin, which not only stimulates hematopoiesis, but also provides immune support to erythrocytes, as well as other blood cells, including the immune system. *Platelets*, which produce the majority of serotonin, can also be classified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



Immunoglobulins or antibodies

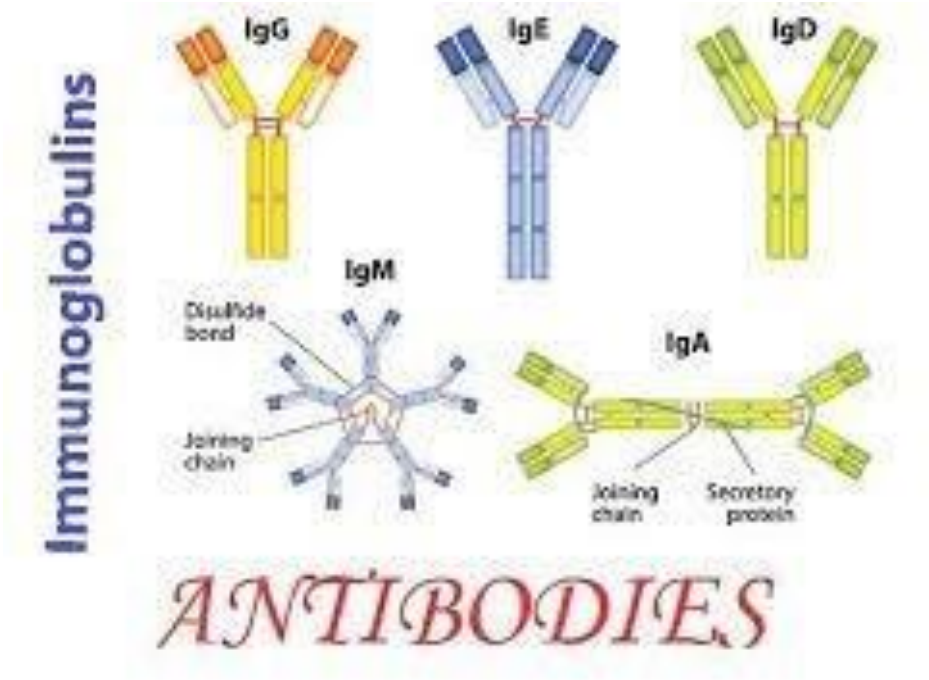
• Cooperation of three cells – macrophages, Th- and Blymphocytes is essential for antibody synthesis.After processing antigens are expressed in cell surface in association with MHC I Iproteins . Th- Lymphocytes produce - IL2 (T-cells growth factor), IL4 (Blymphocytes growth factor) and IL5 (B-lymphocytes 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.



Structure of an Antibody

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.



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.

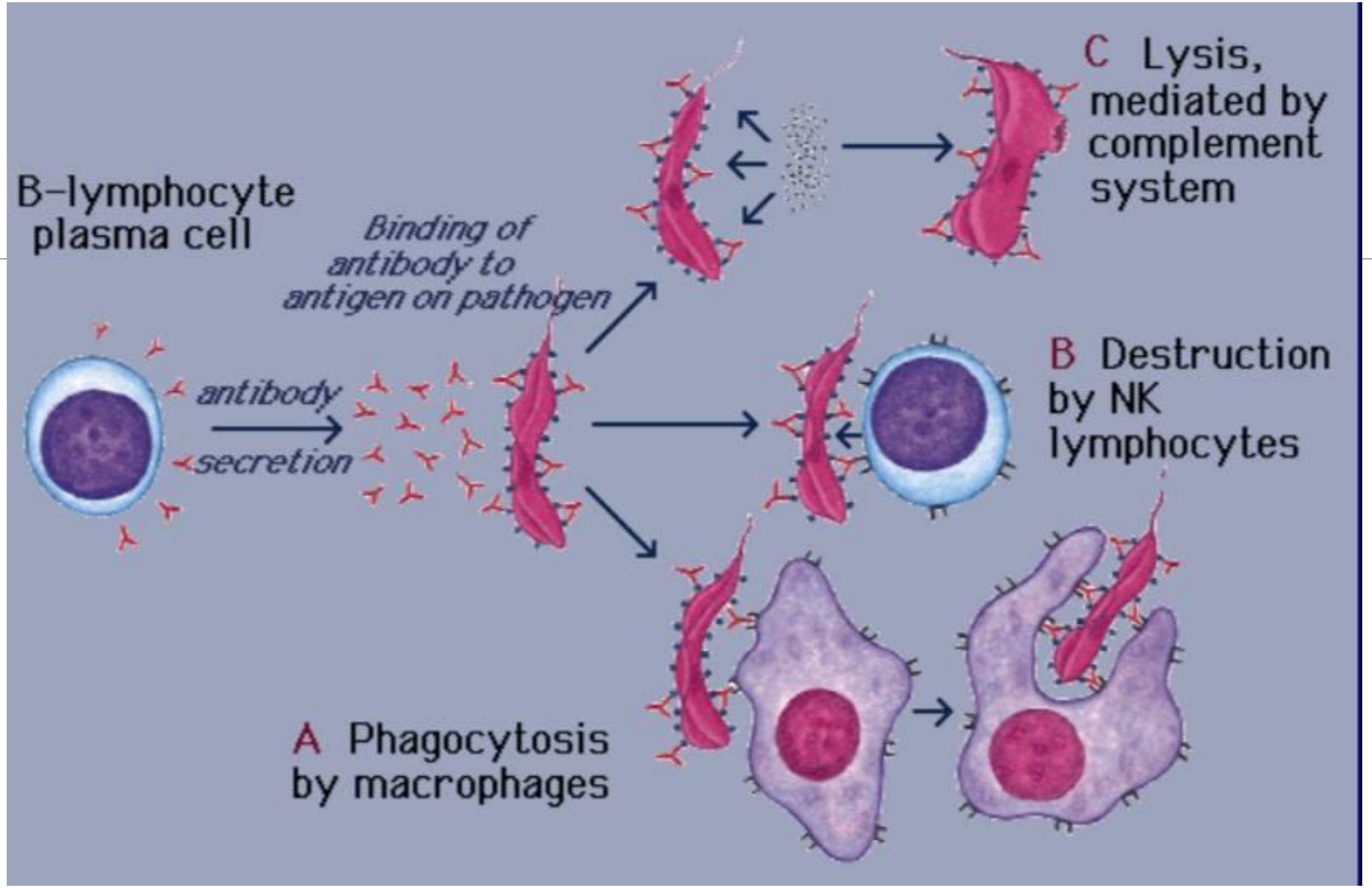
A immunoglobulin (IgA): It makes up 10-15% of immunoglobulins of blood serum. Has two subclasses - IgA1 and IgA2. IgA1 is present in the serum, while IgA2 is a part of sIgA and predominant in the secretions. IgA2 is resistant to the action of proteolytic enzymes of saliva, secretions of intestinal mucosa.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

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 IgMmolecules as well, these immunoglobulins are multimer (dimer and pentamer).

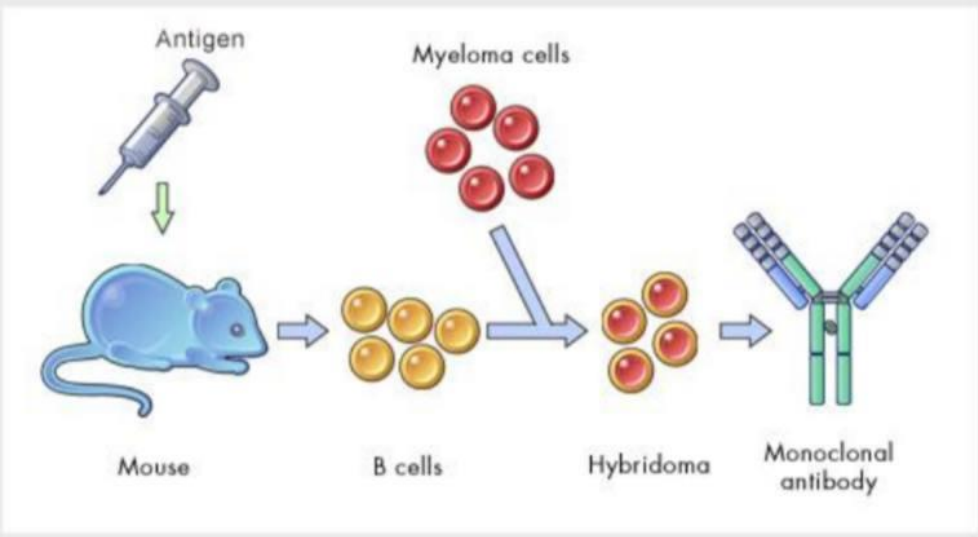
E immunoglobulin (IgE) : It differs from other immunoglobulins by its high cytophilicity - ability to bind to mast cells and basophils. This immunoglobulin has two important biological features:1) it provides immediate type hypersensitivity reactions;2) it is involved in the body's defense response during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

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.

Immunoglobulin 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


B-lymphocyte
plasma cell
antibody
secretion
Binding of
antibody to
antigen on pathogen
C Lysis,
mediated by
complement
system
B Destruction
by NK
lymphocytes
A Phagocytosis
by macrophages

Antibodies function



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

*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.

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.