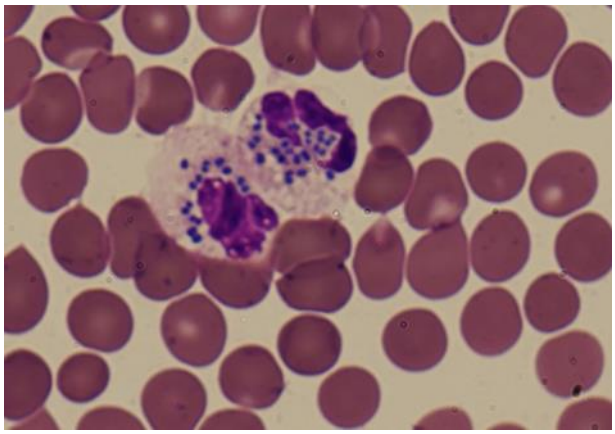
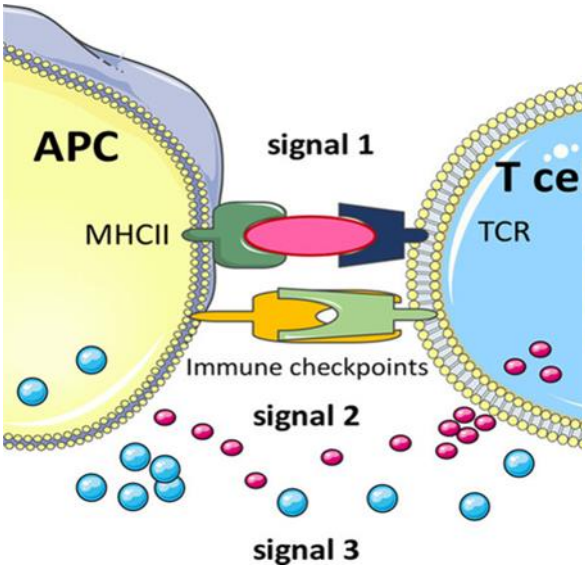
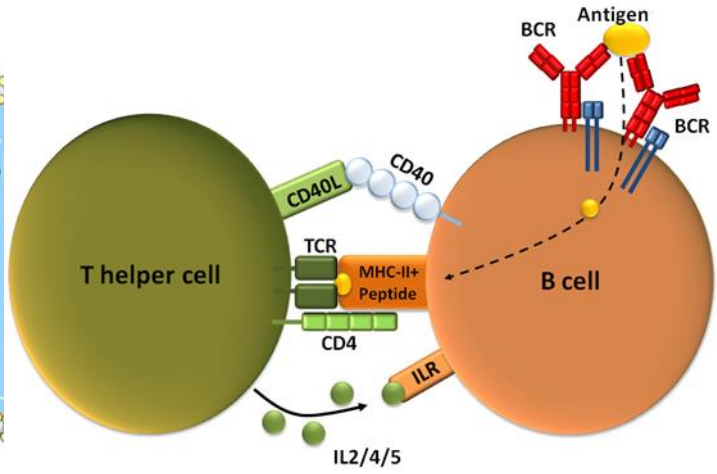


IMMUNOLOGY:
QUESTIONS AND ANSWERS





Міністерство охорони здоров`я України
Українська медична стоматологічна академія

Ганчо О.В., Федорченко В.І., Фаустова М. О.,
Лобань Г.А., Сліпченко Л. Б.

ІМУНОЛОГІЯ: ЗАПИТАННЯ ТА ВІДПОВІДІ

Ministry of Health of Ukraine
Ukrainian Medical Dental Academy

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Посібник для практичних занять з мікробіології, вірусології та імунології для аудиторної та позааудиторної роботи англомовних студентів. Він може бути використаний для підготовки до практичних занять, поточного контролю знань, підсумкового модульного контролю з предмету. Посібник є інтелектуальною власністю і без письмового дозволу авторів не може бути скопійованим і розмноженим у повному обсязі або частинами, окрім рукописної форми. Авторські права захищені Законом України “Про авторське право та суміжні права”.

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Preface

The manual is devoted to one of the most important sections of the general part of medical microbiology - immunity. Knowledge and understanding of the information that the student should study in this section is essential to understand and comprehend many aspects of medical practice. This applies to the pathogenesis of infectious diseases, immunity under conditions of infectious diseases. Knowledge of immunology is a key points to remember for the successful use of drugs for the specific prevention and treatment of infections, as well as for the development of new samplesfor that. There are many techniques, tests of microbiological diagnosis of infectious diseases, which are based on the application of immunological knowledge. In addition, knowledge of immunology is important for understanding the pathogenesis, as well as for the diagnosis and treatment of many diseases of non-infectious nature.

Modern immunology is a branch of knowledge that has accumulated vast amounts of extremely important information that is difficult to comprehend without knowing the basics, the "alphabet" of immunology. Therefore, the authors tried to create a manual that would present in a simple form the basic principles of immunology and cover the full range of issues required for a student of medical higher education to master the subject. The list of sections and subsections of this manual corresponds to the range of issues presented in the Standard program of the subject and are submitted for consideration for practical classes, module control and exams. The knowledge gained from the study of this section is necessary for the successful study of a number of the following disciplines.

In addition, this manual contains a section with a focus on important questions of the immune protection of the oral cavity. This section is primarily intended for dental students, but is also important for students of other faculties.

The team of authors sincerely wishes the readers of this manual a pleasant reading and solid knowledge.

A list of abbreviations

AB – antibody
Ag – antigen
AMI – antibody-mediated immunity
Anti HGG – anti-human gamma globulin
APC – Antigen presenting cells
ASO – Anti-streptolysin O-test
ATS – anti-tetanus serum
APDT – associated based combined pertussis-diphtheria - tetanus - vaccine
AUG – acute ulcerative gingivitis
BCG – bacilla of Calmett and Geren
CBT – complement binding test
CDC – Centers for Disease Control
CFT – complement fixation test
CMC – chronic mucocutaneous candidosis
CMI – cell-mediated immunity
CTL – cytotoxic T-lymphocyte
CNS – central nervous system
DTH – delayed type hypersensitivity
DTaP – diphtheria - tetanus - acelullar pertussis vaccine.
DTP – diphtheria - tetanus - pertussis vaccine
EBV – Epstein-Barr virus
ELISA – enzyme linked immunosorbent assay
IF – immune fluorescence
Ig – immunoglobulin
IL – interleukin
IPS – intra-cellular polysaccharides
IPV – inactivated poliomyelitis vaccine
FAT – fluorescent antibody test
GCF – gingival crevicular fluid
GBS – Guillain-Barré Syndrome
HBsAg – hepatitis B virus s antigen
HBV – hepatitis B virus
HepB – hepatitis B vaccine
HiB – conjugated H.influenza vaccine
HPV – human papilloma virus
HSV – herpes simplex virus

LPS – lipopolysaccharide
MHC – Major histocompatibility complex
MMR – measles - mumps - rubella vaccine
OPV – oral polio vaccine
PCR – polymerase chain reaction
PMC – pseudomembranous candidosis
RBC – red blood cell
RIA – radioimmune analysis
TCR – T cell receptor
TEM – transmission electron microscopy
TNF – tumor necrosis factor

Part 1. IMMUNITY. INNATE IMMUNITY. STRUCTURE OF THE IMMUNE SYSTEM. IMMUNE CELLS

1.1. IMMUNITY AS A WAY OF PROTECTION AN ORGANISM TO SUBSTANCES THAT HAVE SIGNS OF FOREIGNITY AND IS PROVIDED BY A SPECIALIZED IMMUNE SYSTEM. TYPES OF IMMUNITY

The human body has different mechanisms of protection to genetically alien agents. They are divided into two groups – innate responses and specific or adaptive immunity. The human's organism has specialized structures and organs responsible for implementation of immune defence. They are connected with a special system – immune system.

Table 1. Synonyms for terms "innate responses" and "adaptive immunity"

Terms	Synonyms
Innate responses	Nonspecific, innate, species, heritable immunity
Adaptive immunity	Specific, adaptive, individual immunity; immunity

Innate immunity is basic immunity, which may be genetically passed on from one generation to other generation. It does not depend on prior contacts with microorganisms. It may be non-specific when it indicates a degree of resistance to all infections.

Adaptive immunity provides the ability of an organism to resist a particular infectious agent or toxin by recognition and counteraction foreign organisms or substances by the action of specific antibodies or sensitized white blood cells.

Sometimes the term "immunity" can be used to name adaptive immunity only, sometimes – for all mechanisms joined together. It is possible to understand this difference by the context. If the question is about all mechanisms of defence together, it can be classified to some different types (Table 1-2).

Immune system provides some specialized machineries for direct protection of the host, direct fight with everything foreign. There are two groups of factors for both levels of defence - innate responses and adaptive immunity, – molecules, diluted in liquids of the body (humoral factors) and specialized cells (cellular factors).

Table 2. Types of immunity (1)

<table>
<tr><th colspan="4">Immunity</th></tr>
<tr><th colspan="2">Innate</th><th colspan="2">Adaptive</th></tr>
<tr><th>Non-specific</th><th>Specific</th><th>Passive</th><th>Active</th></tr>
<tr><td rowspan="2">It indicates a degree of resistance to all infections</td><td rowspan="2">It shows resistance to particular pathogens</td><td>Natural - provides by transplacental Ig G</td><td>Natural - postinfection (after disease)</td></tr>
<tr><td rowspan="2">Artificial – gamma-globulins in serum drug</td><td rowspan="2">Artificial – postvaccinal</td></tr>
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Table 3. Types of immunity (2)

Level of defense	Humoral	Cellular
Innate responses	Lysozyme, complement system proteins, lysines, leukins, antiviral inhibitors, C-reactive protein, cationic and hydrolytic proteins, lactoferrins, etc. in blood	Phagocytes, natural killer cells
Adaptive immunity	Antibodies	T-cytotoxic (effectors)

Table 4. The differences between innate responses and specific immunity

Innate responses	Specific immunity
Response is antigen-independent	Response is antigen-dependent
There is immediate maximal response	There is lag time between exposure and maximal response
Non antigen-specific	Antigen-specific
Exposure results in no immunologic memory	Exposure results in immunologic memory

In addition, immune system cells produce a lot of biologically active substances, which are necessary for defence, working with foreign agent indirectly. Another levels of cells - nonspecific and specific – are producers of these regulative molecules.

1.2. FACTORS OF INNATE IMMUNITY

Innate responses have a lot of different mechanisms. Many of them, but not all, are connected with activity of immune system directly. Innate responses can be divided into 2 lines of defence. The third one is line of specific defence.

The first line of defence
(not connected with activity of immune system directly)

Epithelial surfaces

External physical barriers which restrict entry of pathogen. Examples: skin, mucous membranes and hair, blood clotting. The intact skin and mucous membrane covering the body gives considerable protection against bacteria on it. They provide mechanical barriers. They also provide bactericidal secretions (for second line).

Movement out of body. Examples: respiratory tract ciliated epithelium (moves particles trapped in mucus toward the pharynx), bleeding, urinating, vomiting, diarrhea, tears, coryza.

Chemical defence. Examples: the wax in the ears, the sticky mucus lines of nose and other openings, the acid in the stomach.

Normal microflora

Intact normal flora realises the protection of the colonized surfaces in a healthy host body. It has been discussed in several contexts.

There are three maine ways that the normal flora protects the surfaces:

1. **Competition with non-indigenous species** for binding (colonization) sites. The normal flora is highly-adapted to the tissues of their host;
2. **Nonspecific antagonism to non-indigenous species**. The normal flora produces a variety of metabolites and ends products that inhibit other microorganisms. These include fatty acids (lactate, propionate, etc.) and peroxides;
3. **Specific antagonism to non-indigenous species**. Members of the normal flora may produce highly specific proteins called bacteriocins, which kill or inhibit other (usually closely-related) species of bacteria.

Areactivity of cells and tissues

This concept is about specific innate immunity (see Table 1-2). Areactivity of cells and tissues is connected with **absence of cells receptors for pathogen** absorption or toxins fixation. A pathogen should attach to some cells surfaces by

specific receptors of the host. Such interaction is necessary for next stage - multiplying of microbe. It means disease is not possible without attachment.

In another situation it can be because of **cellular machinery has not the needs of the microorganism.**

In the both examples the question is that the pathogen of one species can be not pathogenic for another one.

The second line of defence
(connected with activity of immune system directly)

Innate non-specific immune defences provide rapid local response to pathogen after it has entered host (tissue factors). If the barrier of body is overcomed by the microorganisms, a number of factors plays its role in normal tissue and body fluid. Tissue factors may be divided into: cellular and humoral factors.

Examples:

Cellular factors - leukocytes (phagocytes: macrophages and neutrophils), basophils, eosinophils; lymphatic system.

Humoral factors - organic substances present in the extracellular fluids of the body - complement cascade responses, interferon responses, lysozyme in tears, swear and saliva; lysines, leukins, antiviral inhibitors, C-reactive protein, cationic and hydrolytic proteins, lactoferrins, etc. in blood.

Fever and inflammatory responses are provided by interaction of the cellular and humoral factors.

The third line of defence

Antigen-specific immune responses specifically target and attack invaders that go through first two lines of defence. Examples: **antibodies and lymphocytes**.

1.3. STRUCTURE OF THE IMMUNE SYSTEM. CENTRAL AND PERIPHERAL ORGANS OF THE IMMUNE SYSTEM

The immune system (lymphatic system) is a defence system against pathogens. It is incorporated into every system of the body. The immune system has its special vessels (lymph vessels) that run through the body. White blood cells are one of the major parts of the immune system.

The immune system has:

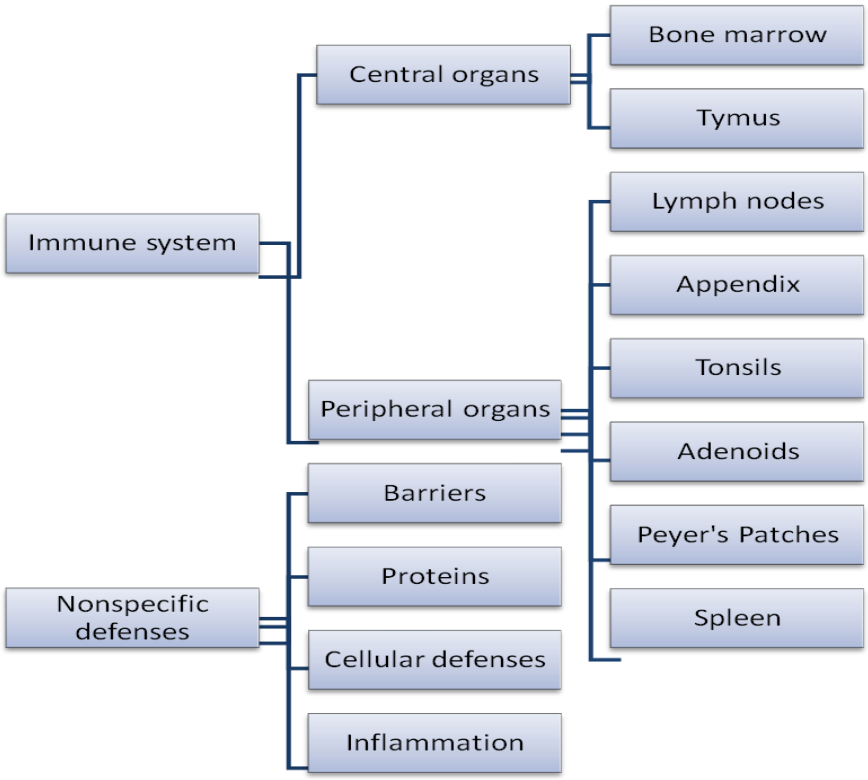

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Figure 1. Immune system

Central organs include bone marrow (all WBC source) and thymus (is the placement of the T-cells proliferation).

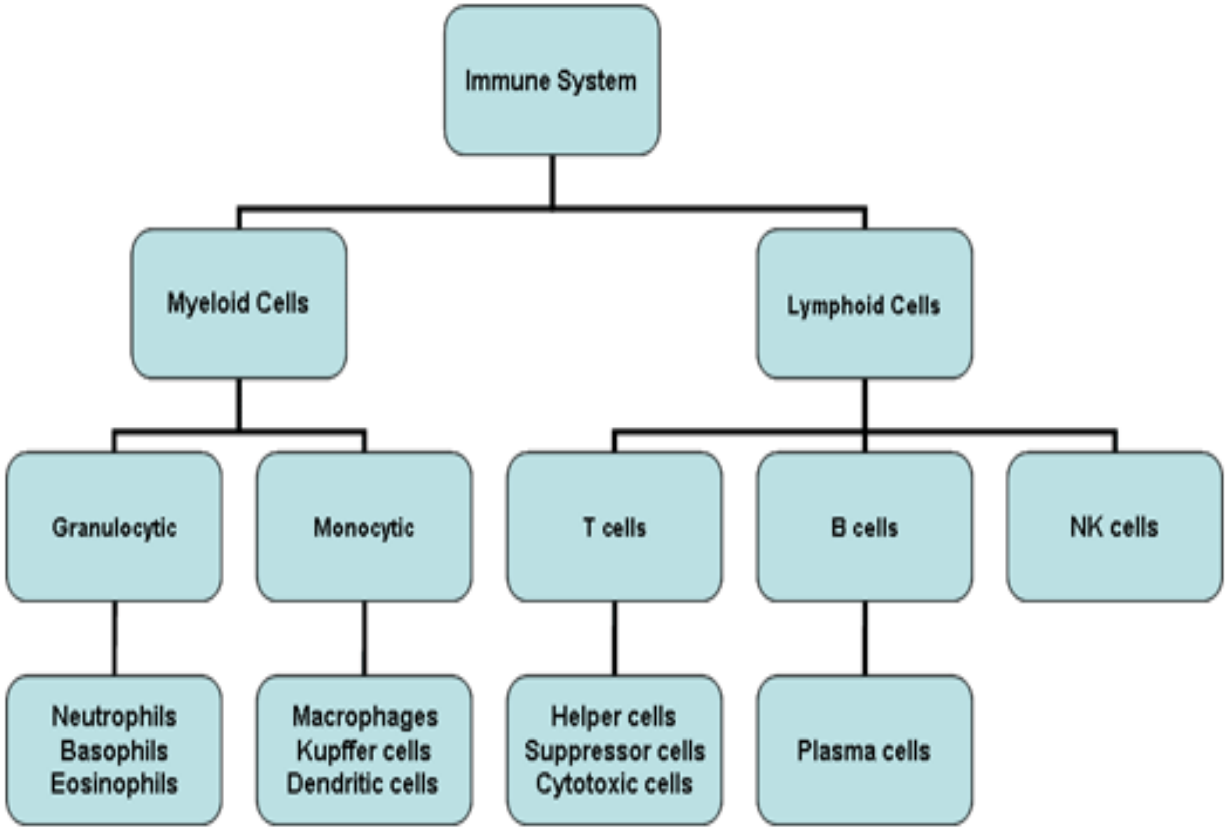
Peripheral organs include Lymph nodes, Peyer's Patches, Adenoids, Tonsils, Spleen.

The immune system has been divided into two levels by the criteria of immune cells interaction with foreign substances (antigens). Early, antigen-independent stages have place in the central organs, while contact with antigens and maturation of immune cells occurs in peripheral organs. After that, immune cells are ready to defend.

The immune system is closely connected with the circulatory system. White blood cells are present in the immune system, blood and tissues.

1.4. IMMUNE CELLS. SURFACE MARKERS (ANTIGENS) AND RECEPTORS OF THESE CELLS

The white blood cells are represented on the Figure 2. There are cells of innate responses and specific defence, humoral and cellular directions.



http://www.microbiologybook.org/ghaffar/innate-back.htm

Figure 2. Immune cells origin

Lymphocytes

Lymphocytes (lymphoid cells) are one of the principal leukocytes. There are three major types of lymphocytes B, NK and T. The T- and B-cells in the lymph nodes are confined to discrete zones. Thay have distinctive surface markers and functions.

B cells are called so because they are connected with a special organ “bursa” in birds. Mature B cells (**Plasma cells**) are the producers of antibodies or immunoglobulins - Ig (classes A, M, G, E and D). **B-memory cells** are capable for reacting in future. It is the essence of the humoral adaptive immunity.

Additionally, B cells present antigens (they are also classified as professional antigen-presenting cells (APCs).

NK (normal killer cells) carry out extracellular killing of the target cell – tumor cells or virus-infected cells with antibody-independent cytolytic granule

mediated or cytokine-induced NK activated cell apoptosis. It is possible for antibody-dependent cytotoxicity resulting in NK activation, release of cytolytic granules and consequent cell apoptosis. Antibody plays here recognizing role while the NK-cell is affecting participants. NK cells are representatives of innate cellular immunity that distinguishes them from another lymphocytes.

T cells are called so because they are predominantly produced in the thymus. T cells are factors of cellular adaptive immunity. The main of them are **T cytotoxic (CD8 T cells) and CD4 T cells.**

T cytotoxic cells are the effector cells which recognize specific antigen on the surface of the target cell and after attachment kill it extracellularly. The special proteins with enzymatic activity (perforins) are released by the cell for that. Perforins destroy cellular membrane.

Myeloid cells

First subgroup of this is granulocytic cells (**granulocytes**). There are **neutrophils, basophils, mast cells and eosinophils**. All these types of cells work on innate cellular area of defence. Their activity can be enhanced by feedback with adaptive level of immunity (by opsonins and IgE).

Neutrophils (microphages) are cells with phagocytic activity. They are present in blood and tissues. They migrate in injured zones very fast and take part in the inflammation reactions.

Basophils and mast cells release histamine, provide allergic response, are antiparasitic.

Eosinophils are involved in parasite defence and allergic response.

The second subgroup is **monocytic cells (macrophages).** This group of cells defends at the innate cellular level. In contradistinction to neutrophils, macrophages are directly connected with adaptive level of defence (with CD4 T cells). The macrophages are the main **antigen-presenting cells (APCs).** It has place in **process of co-operation** between immune cells in the process of forming an adaptive immune response.

Membrane markers (antigens) and receptors

There are a lot of specialized structures on the surfaces of immune cells. The functions are in direct and indirect interaction (co-operation) between immune cells in the immune response formation and realizing. Additional group of receptors are specific receptors for foreign antigens recognition. Some of them are typical of special populations and subpopulations of immune cells, so it can be used to reveal or to count them for some aims. This surface sructures are named markers of the immune cells.

Membrane markers and receptors on B-cells and T-cells

The nature of the membrane receptors for antigen on B-cells and T-cells is fairly well understood. Each B-cell has approximately 10^5 membrane-bound antibody molecules (IgD or IgM), which correspond in specificity to the antibody that the cell is programmed to produce.

Each T-cell has about 10^5 molecules of a specific antigen-binding T-cell receptor (TCR) exposed on its surface. The TCR is similar, but not identical, to antibody.

In addition, T-cell subsets bear some distinguishing surface markers, notably **CD4** or **CD8**. T-cells bearing **CD4** always recognize antigens in association with class **II MHC** proteins on the surfaces of other cells.

T-cells bearing **CD8 (CD8)** always recognize antigen in association with class **I MHC** proteins and typically function as **cytotoxic T-cells**.

Table 5. Immune cells

Cells	Characteristics	Markers	Functions
Natural cytolytic cells			
Natural killer cells	Large granular lymphocytes	Fc receptors for antibody; CD16, CD56, CD57	Kill antibody-decorated cells and virus-infected or tumor cell
Phagocytic cells			
Neutrophils (polymorpho-nuclear leukocytes)	Granulocytes with short life span, multilobed nucleus and granules, segmented band forms	-	Phagocytise and kill bacteria
Eosinophils	Bilobed nucleus, heavily granulated cytoplasm	Staining with eosin	Are involved in parasite defence and allergic response
Macrophages	See below	-	-

Antigen-presenting cells (APCs)			
Monocytes	Found in lymphocytes, blood, lungs, and other organs	Horseshoe-shaped nucleus, lysosomes	Are precursors to macrophage-lineage, lymphokine release
Macrophages	Possible residence in tissue, spleen, lymph nodes, and other organs	Large, granular cells; Fc and C3 receptors	Initiate inflammatory and acute phase response; activated cells have antiviral, antibacterial, and antitumor activities
Langerhans' cells	Presence in skin	-	Transport antigen to lymph nodes
Dendritic cells	Alpha lymph nodes, tissue	-	Are efficient antigen presenter
Microglial cells	CNS and brain	-	Produce cytokines
Kupffer's cells	Presence in liver	-	Filter particles from blood (e.g., viruses)
B cells	See below	-	
Antigen-responsive cells			
T-cells	Mature in thymus; large nucleus	CD2, CD3, T-cell receptor	Produce lymphokines
CD4 T-cells	Helper / DTH cells; activation by LPC through class II MHC antigen presentation Th 1 subtype Th 2 subtype	CD2, CD3,CD4, T-cell receptor, IL-2, IFN-gamma-production IL-4,5,6,10-production	Produce lymphokines; stimulate T- and B-cell growth; promote B-cell differentiation, antibody production Promote initial defenses (local), DTH, T cytotoxic cells. Promote humoral response

CD8 T-cytotoxic cells	Recognition of antigen presented by class I MHC antigens	CD2,CD3,CD8, T-cell receptor	Kill viral, tumor, non-self (transplant) cells; secrete TH1 lymphokines
CD 8 T-cells (suppressor cells)	Recognition of antigen presented by class II MHC antigens	CD2,CD3,CD8, T-cell receptor	Suppress T- and B-cell response
Antibody-producing cells			
B-cells	Mature in Peyer's patches, bone marrow, bursal equivalent; large nucleus; activation by antigens and T-cells	Surface antibody, class II MHC antigens	Produce antibody and present antigen
Plasma cells	Small nucleus, large cytoplasm	-	Are terminally differentiated, antibody factories
Other cells			
Basophils/ mast cells	granulocytic	Fc receptors for IgE	Release histamine, provide allergic response, are antiparasitic

Notes: MHC – Major histocompatibility complex; TNF – tumor necrosis factor; CNS – central nervous system; DTH – delayed type hypersensitivity; IL – interleukin; Ig – immunoglobulin; LPS – lipopolysaccharide

1.5. PHAGOCYTOSIS, THE NOTION OF OPSONINS. CLASSIFICATION OF PHAGOCYTIC CELLS. BASIC STAGES OF PHAGOCYTOSIS. COMPLETED AND INCOMPLETED PHAGOCYTOSIS

Phagocytosis is the process of ingestion and intracellular destroying of bacteria or other foreign particles if invasion of blood and tissue has place. It is natural defence against pathogens mediated by phagocytic cells. The phagocytosis can be completed and incompleted.

Opsonines

Opsonines are substances, which improve the effectiveness of phagosytosis by marcing the antigen. There can be antibodies, complement proteins and circulating proteins.

Phagocytic cells

Microphages, e.g. polymorphonuclear leucocytes, or neutrophils;

Macrophages include:

1. **Histiocyts** (wandering amoeboid cells in tissue);
2. **Fixed reticuloendothelial cells** (in the reticuloendothelial system – RES, see next chapter)
3. **Monocytes** (in blood);
4. **Dendritic cells** (are present in those tissues that are in contact with the external environment, such as the skin (where there is a specialized dendritic cell type called the **Langerhans cell**) and the inner lining of the nose, lungs, stomach and intestines. They can also be found in an immature state in the blood);

B-lymphocytes– these cells require some phagocytosis to transform into antibody producing plasma cells.

The process of the completed phagocytosis consists of next stages.

Table 6. Stages of completed phagocytosis

Stage	Characteristics
Activation by inflammatory mediators	Activators are bacterial proteins, capsules, peptidoglycan, prostaglandins, complement proteins.
Chemotaxis	This is the directional movement of the phagocyte towards a chemical attractant (bacterial products (e.g.

	endotoxin), injured tissues, complement proteins (C3a, C4a, C5a) and chemical substances produced by leukocytes (leukotrienes)
Margination, Rolling and Adhesion	Leukocytes assume marginal positions in the blood vessels, stick to the walls of the venules and roll along them until they become firmly attached to the vessel wall
Diapedesis	Leukocytes go out of the venules
Recognition-Attachment	It can be done due to innate (unenhanced) way or through the enhanced rout with participation of complement molecules (C3b, C4b) or antibody molecules (IgG). This factors of enhancement is named “opsonins” (opsonization is enhanced attachment).
Phagocytosis	The phagocyte grabs the microbe into a phagosome and it fuses with a lysosome to form a phagolysosome. Lysosomes contain a lot of enzymes which can destroy the pathogen. There are two ways of pathogen killing: The oxygen dependent pathway (oxidative burst) - more effective mechanism by reactive oxygen molecules such as the superoxide radical and hydrogen peroxide. The oxygen independent pathway (less effective mechanism). It is due to destruction of the pathogen via lysosomal enzymes such as proteases, phospholipases, nucleases and lysozyme.
Exositosis	There are soluble substances that release from the cells. Может так

Incompleted phagocytosis is characterised with no effective killing and digestisng of microorganisms. The engulfed pathogen is protected in phagocytic cells from another factors of defence or antibiotics in the body fluids. Some pathogens can multiply in phagocytic cells, kill phagocytic cells to be spreaded in phagocytic cells through the lymph or blood to other parts of the body, causing widespread infection.

1.6. THE SIGNIFICANCE OF PHAGOCYTOSIS IN PROVIDING NATURAL IMMUNITY AND THE DEVELOPMENT OF THE IMMUNE RESPONSE. METHODS OF PHAGOCYTIC ACTIVITY ASSESSMENT: PHAGOCYTIC NUMBER, PHAGOCYTIC INDEX

Phagocytosis has important significance in the implementation of natural immunity and the development of the specific immune response. At first, this is cellular factor of the innate immunity. Then phagocytosis is the first step in co-operation between immune cells in the process of the specific immune response formation by direct contact with lymphocytes and by production of numerous humoral factors with biological activity. As a result, phagocytosis proceeds more vigorously in the immune than in the non-immune organism.

Table 7. Assessment of phagocytosis efficiency

Number of phagocytic neutrophils	Number of "empty" neutrophils	Number of engulfed by neutrophil particles		
		1-10	11-20	21 and more
a	b	c	d	e

Determination of phagocytic activity

It is based on the ability of phagocytes to engulf latex particles, which are stained by Romanovsky-Giemza in blue. 200 leukocytes are seen with the microscope use and the number of engulfed particles is determined (Table 7). **Percent of neutrophils with phagocytic activity is the <u>phagocytic number</u>.**

<u>The phagocytic (opsonic) index</u> (x) is the number of engulfed particles in one neutrophil. It is calculated with formula use: $X = \frac{5c + 15d + 25e}{a}$, where 5, 15, 25 are the middle numbers of engulfed particles in one neutrophil; c, d, e – number of neutrophils with phagocytic activity.

1.7. HUMORAL FACTORS OF INNATE RESPONCES PROTECTION: COMPLEMENT SYSTEM, LYSINES, INTERFERONS, LEUKINS, ANTIVIRAL INHIBITORS, AND LYSOZYME. METHODS OF THEIR ASSESSMENT

Complement system

It is the complex set of blood proteins, which consists of over 30 fractions, each of which has a certain property. Most of the proteins and glycoproteins that constitute the complement system are synthesized by hepatocytes, tissue macrophages, blood monocytes, and epithelial cells of the genitourinary system and gastrointestinal tract.

After stimulation, the system cleaves specific proteins to release cytokines and initiate an amplifying cascade of further cleavages.

There are different pathways of complement system activation. It may be activated in a cascade in 3 pathways: **classical, alternative and lectin** (MBL-mediated by lectin).

Complement system, also known as **complement cascade**:

1) causes lysis of microbes and other cells by the cell-killing **membrane attack complex (MAC)**;
2) takes part in specific immunological reactions and virus neutralization;
3) intensifies phagocytosis, chemotaxis and inflammation.

Table 8. Complement pathways

Pathway	Activation by:
Alternate or properdin	Bacteria and bacterial products
Lectine	Lectin binding to sugars on the microbe surface
Classical **(most effective)**	Complexes of antibody and antigen

After binding to activator, every fraction, to be activated, cleaves to 2 or more subunits. Some of it is soluble and biologically active. One of the released parts from each complement fraction sequentially attaches to the chain on the surface of the activator. All three pathways finally coalesce in "the main" fraction – C3. Next fraction always is C5. Next part of the chain with C6, C7, C8 and C9 is the "**membrane attack complex" (MAC).**

Interferons

Interferons (IFNs) are group of low-molecular-weight protein (cytokine) that carry out control and regulatory functions aimed at preserving cellular homeostasis.

The most important of these functions are **antiviral, antitumor, immune-modulating, antibacterial and radioprotective.**

IFNs interact with different cellular receptors. Interferon is subdivided into three types: interferon-α (from leukocytes), interferon-β (from fibroblasts) and interferon-γ (lymphocytic, or immune). Induction of interferon synthesis may be caused by viruses, bacteria, fungi, plant extracts, and synthetic compounds, various drugs, radiation, etc.

IFNs are applicated in therapy several neoplastic and not neoplastic diseases.

Tumor necrosis factor

Tumor necrosis factors (TNF family) is a group of cytokines which are mainly secreted by macrophages and can induce cell death of certain tumor cell lines.
It is a soluble inflammatory cytokine that is found in all mammalian tissues. Tumor necrosis factor plays important roles in diverse cellular events such as cell survival, proliferation, differentiation, and death. It may be involved in inflammation-associated carcinogenesis.

Basic (cationic) peptides

Defensins and cathelicidins are bactericidal substances active at high pH (7 to 8). They act upon cell wall causing cell disintegration. These can kill bacteria. Fungi, and inactivate viruses.

Lysozyme

It is bactericidal enzyme found in nasal and intestinal secretion, seminal fluid and lacrimal secretion. It can destroy peptidopolysaccharide of grampositive bacteria cell wall, which consists of 90 % murein. Lysozyme is synthesized by macrophages and provides bactericidal properties of blood, saliva, and mucosa.

Beta lysine

It is relatively thermostable (destroyed at 65-70^{o}C) bactericidal substance active against anaerobes and aerobic spore-forming bacteria (anthrax bacillus). It is liberated from platelets during clotting.

C-reactive protein (CRP)

It is precipitate when mixed with somatic polysaccharide C of pneumococcus in presence of calcium ions. These nonspecific substances appear in blood of a person with tissue necrosis and inflammation. C-reactive protein does play an important role in the resolution of inflammatory process. Recently, it is suggested that the functions CRP are primarily to act as a binding mechanism, say for pneumococcal "C"-

polysaccharide is most effectively precipitated or agglutinated. The binding of such ligands render them accessible to phagocytosis and as a result to clearance and metabolic breakdown.

Bactericidin

It is nonspecific serum factor active against Neisseria, Streptococcus haemolyticus, etc.

Non-specific hyaluronidase inhibitors

It is tissue damage nonspecific inhibitor hyaluronidase that appears in blood. It is heat labile and requires magnesium ion for its activity.

Inhibitors of viral activity

They are the first humoral barrier that prevents virus contact from the susceptible cells. Thermostable inhibitors can inactivate infectious, toxic, haemagglutinate properties sensitive to inhibition of viruses strains. Thermostable inhibitors can block connections of virus with the host cell receptors. People with high levels of inhibitors in the blood have a greater resistance to viral infections. **Acute phase proteins**

A large group of proteins that is produced in a body during inflammatory responses after infection or injury, during ontogenecity, pregnancy and have antimicrobial action, promotes phagocytosis, complement activation, the formation and elimination of inflammation. The bulk of acute phase proteins consists of C-reactive protein, serum amyloid A and P. Other acute phase proteins - are blood coagulate factors, metallic-binding proteins, protease inhibitors and some components of complement.

Part 2. ANTIGENS AND ANTIBODIES. ADAPTIVE IMMUNITY

2.1. FORMS AND TYPES OF IMMUNE RESPONSE

The immune system distinguishes two groups of foreign substances. One group consists of antigens that are placed outside the host cells. These antigens include molecules, viruses, and bacterial cells. A second group consists of self cells that display aberrant MHC proteins. Aberrant MHC proteins can originate from antigens that have been engulfed and broken down (exogenous antigens) or from virus infected and tumor cells that are actively synthesizing foreign proteins (endogenous antigens). **Depending on the kind of foreign invasion**, two different immune responses occur: **humoral and cellular.**

The immune response is a chain of successive complex cooperative processes going on in the immune system in response to the action of the antigen in the body. Cells, involved in the immune response (T and B lymphocytes and APC) are called immune or immunocompetent cells. The immune response to antigens of microbial origin underlies the infectious immunity.

According to the type of interaction of antigen and formed effector cells (**according to the final result**), it is customary to distinguish next types of immune response:

1) humoral immune response,
2) cellular immune response,
3) mmunological memory
4) immunological tolerance.

2.2. ANTIGENS AS INDUCERS OF IMMUNE RESPONSE. STRUCTURE OF ANTIGENS, CLASSIFICATION

Antigens -Ag - (immunogens) are any substance that is genetically foreign to the human host and can induce immune response. Antigen then is capable of reacting with the products of a specific immune response, e.g., antibody or specific sensitized T-lymphocytes.

Antigenic determinants (epitopes)

The active sites are present at certain places in antigen molecules. These active sites are called antigenic determinants or epitopes.

Antigenic substances have such properties:

1. Colloid structure;
2. Solubility in the body fluids.

Completed antigens

Chemical structure of a complete antigen is **protein**. Antigens include bacteria, viruses, rickettsia or fungi that cause infection and disease.

Antigenic properties are pertinent to toxins of a plant and an animal origin, enzymes, native foreign proteins, various exogenous cellular elements of tissues and organs.

All of this examples are named "**exogenous antigens**" because the source they come from is outside the body.

Incompleted antigens

Incompleted antigens (haptens) are low molecular weight substances. These substances are not immunogenic by itself. If it joins to a larger carrier molecule (albumin, globulins), they become immunogenic. Examples: simple chemicals and drugs - penicillin, sulphonamid, aspirin, cosmetic, tranquillizers, neomycin skin ointment. If incompleted antigens are connected with appropriate **carrier molecule**, it can be immune response like to completed antigen.

Endogenous antigens

Endogenous antigens source is the host's body. There are two maine groups of endogenous atigens: autoantigens and tumor antigens.

Autoantigens

An **autoantigen** is usually a normal protein or protein complex (and sometimes DNA or RNA) that is recognized by the immune system of patients suffering from an autoimmune disease. It can be connected with disorder of a neurohumoral regulation of immune homeostasis.

Tumor antigens

Tumor antigens are presented by tumor cells and normal cells (so called tumor-associated antigens – TAAs). Cytotoxic T lymphocytes that recognize these antigens may be able to destroy tumor cells. Tumor antigens can appear on the surface of the tumor in the form of a mutated receptor, in this case they are recognized by B cells.

Novel peptides (neo-epitopes) for human tumors without a viral etiology are created by tumor-specific DNA alterations.

Superantigens

Superantigens (SAgs) are active at very low concentration causing release of large amounts of cytokines. They have the ability to bind both class II MHC molecules and TCR β chain. It causes the massive T-cell activation and release of large amounts of cytokines cause systemic toxicity. This method of stimulation is not specific for the pathogen. Superantigens not lead to adaptive immunity (no memory). They activate multiple clones of T-lymphocytes. It causes inflammation.

Examples of the superantigens are some bacterial toxins:

1. S. aureus toxic shock syndrome toxin (TSST) and enterotoxins;
2. S. pyogenes pyrogenic toxin A.

The long time superantigen presence in a body leads to immunodeficiency because of exhaustion of the lymphocyte clones.

Factors that influence immunogenicity

1. Foreign substances are immunogenic.
2. Proteins are more immunogenic, lipids and polysaccharides are less.
3. Molecular size: high molecular weight increases immunogenicity.
4. Chemical structure complexity: high complexity increases immunogenicity.
5. Route of administration: parenteral route is more immunogenic then oral route.
6. Antigen dose:
7. Appropriate dose - optimum antigenicity
8. Low dose – low - zone tolerance
9. High dose - high-zone tolerance
10. Adjuvant: Substance when injected with an antigen enhance immunogenicity.

Antigen specificity

Antigen specificity is of the following types:

1. **Species specificity**: Tissue of all individuals in species contain species specific antigen. It has been useful in tracing of evolutionary relationship between species and in identification of species of blood and seminal stains when a forensic application occurs.
2. **Allospecificity (isospecificity):** an antigen is presented only in some individuals (as of a particular blood group) of a species and capable of inducing the production of an alloantibody by individuals which lack it.
3. **Organ specificity:** They are restricted to particular organ or tissue of species. When they are restricted exclusively to an organ they are called organ specific.
4. **Heterogenetic specificity:** This is found in a number of unrelated animals and microorganisms. The examples are:

a) Forssman antigen found in the tissue of guinea pigs, cat, horse, sheep, bacteria, e.g. rickettsia.
b) Weil Felix reaction in typhus fever.
c) Paul Bunnel reaction in infectious mononucleosis.
d) Cold agglutinin in primary atypical pneumonia.

5. **Auto specificity**: a number of tissue antigens may act as autoantigens, e.g. lens protein, thyroglobulin, etc.

2.3. ANTIGENIC STRUCTURE OF MICROORGANISMS. LOCALIZATION, CHEMICAL COMPOSITION AND SPECIFICITY OF BACTERIAL AND VIRAL ANTIGENS, ENZYMES, TOXINS. THE ROLE OF MICROBIAL ANTIGENS IN THE INFECTIOUS PROCESS AND THE DEVELOPMENT OF THE IMMUNE RESPONSE.

To fight with pathogen, immune system should recognize its antigens. It is important to remember that immune system factors can interact with antigens directly only. It is why us antigens in bacterial cells are important their surface structures end produced substances (exotoxins).

Antigens of bacterial cells

Antigens related to bacterial cells are named by their location only, even if these are different chemical composition.

Somatic antigen (O): cell wall structures. For Gram-negative bacteria it is lipopolysaccharide composition (LPS), for Gram-positive it is more peptidoglycan.

Capsular antigen (K): for capsulated bacteria only. Often it is polysaccharide composition, sometimes protein.

Flagellar antigen (H): in flagella. It consists of protein – flagellin.

Fimbrial antigen (F): surface antigens in fimbria of bacilli;

Antigen secreted by bacteria: exotoxin. These are always biologically active proteins.

Viral antigens

It is the same for **viral antigens** – there are important their surface structures. Glycoprotein coat viral antigens **(spikes or peplomers)** are important for recognition of free virions and viral antigens on the surface of infected cell. The antigens of influenza virus are **Hemagglutinin** and **Neuraminidase**.

Surface microbial structures have important role in infectious diseases. From one side, microorganisms are coated with incomplete antigens to prevent strong immune response of the host. Proteins are present only if it is necessary for implementation some functions. It can be for attachment to target cell (fimbria of bacteria, viral gemagglutinin), movement (flagella), releasing out from the host cell (viral neuraminidase), damaging of some host's structure or process (exotoxins).

From another side, pathogens are recognized with immune system by their antigen structure. Accordingly, to efficiency of the interaction of pathogen and immune system, it can be different properties of immune defence and different result of the defence. It can be short or long time of disease, short or long time of the immune memory. Sometimes it can be present even negative result for host in the immune activity (for example, allergic reaction or activity against own cells).

2.4. CO-OPERATION OF IMMUNE CELLS IN THE ADAPTIVE IMMUNE RESPONSE

Stages of the co-operation

The stages of the co-operation are:

1. Phagocytosis by antigenpresenting cells
2. Processing
3. Presentation to lymphocytes
4. Clonal proliferation of lymphocytes
5. Maturation of lymphocytes
6. Effector activity of lymphocytes (antibody production by B-lymphocytes, cytotoxisc aktivity of T- cytotoxic cells).

Mechanisms of immune cells co-operation

The co-operation of immune cells has some different mechanisms:

1. Distant interaction of the immune cells by soluble substances (**cytokines**) with selective biological activity;
2. Direct interaction of the immune cells by special tools (proteins of the **Major Histocompatibility complex** – MHC);
3. **Direct specific interaction** of the immune cells.

All these directions are inextricably linked and are working together.

2.5. IMMUNE CELLS INTERACTION IN THE PROCESS OF IMMUNE RESPONSE. CYTOKINES

Cytokines and their role in the formation of immune response

Cytokines are the soluble mediators (like hormons) of host defence responses. Cytokines are produced by multiple cell types and can produce multiple effects on the same cell, and they can act on many different cell types. Cytokines are the peptides or glycoproteins. As regulators of cytokines production there may be another cytokines, hormones, prostaglandins, antigens and many other agents, which can act to the immune cell.

Better understanding the functions, reciprocal regulation, and counterbalance of subsets of immune and inflammatory cells that interact through **interleukins, interferons, tumor necrosis factor alpha (TNF-a), and transforming growth factor beta (TGF-b)** offer opportunities for immune treatment in the era of development of biological immune response modifiers particularly targeting these molecules or their receptors.

More than 60 cytokines have been designated as interleukins since the initial discoveries of monocyte and lymphocyte interleukins (called IL-1 and IL-2, respectively), their cellular sources, targets, receptors, signalling pathways, and roles in immune regulation.

Cytokines are cell signalling molecules that aid cell to cell communication in immune responses and stimulate the movement of cells towards sites of inflammation, infection and trauma The term "cytokine" is derived from a combination of two Greek words - "cyto" meaning cell and "kinos" meaning movement.

Cytokines exist in peptide, protein and glycoprotein (proteins with a sugar attached) forms. The cytokines are a large family of molecules that are classified in various different ways due to an absence of a unified classification system. Cytokines produced cells are not immune system cells only. It can be endothelial cells and fibroblasts too.

Principles of cytokines action

1) if a cell-producer is stimulated accordingly, it starts to produce its cytokine;
2) the cytokine akts to its target cell through a special surface receptor;
3) this interaction leads to some biochemical reaction;
4) it works as a sign sent to the cell machinery;
5) the target cell performs its inherent function –effector and (or) regulator (really both). It means the cell defends and produces next cytokins to activate next cells.

Table 9. Cytokines and cytokine produced cells

Substances / Produssers	Monokines	Lymphokines	Interleukines	Cytokines
Produssers	Monocytes, Tissue macrophages	T-lymphocytes (NK-cells, CD4 cells, T- cytotoxic, T-supressers, B-cells, Plasma cells	All for Monokinse and Lymphokines	All for Interleukins and endothelial cells and fibroblasts substances

These interactions by cytokins are the direct and the reverse to balance directions and efficacy of the immune difence on innate and aquied levels.

Examples of cytokines include the interleukins and the interferons, which are involved in regulation the immune system's response to inflammation and infection.

The most current terminology used to describe cytokines is "**immunomodulating agents**" or agents that modulate or alter the immune system response. Cytokines are important regulators of both the innate and adaptive immune response.

Interleukins

Interleukins are a group of cytokines (secreted proteins and signal molecules) that were first seen to be expressed by white blood cells (leukocytes). The term interleukin derives from (inter-) "as a means of communication", and (-leukin) "deriving from the fact that many of these proteins are produced by leukocytes and act on leukocytes". The function of the immune system depends on a large part on interleukins, and rare deficiencies of a number of them have been described, all featuring autoimmune diseases or immune deficiency. The majority of interleukins are synthesized by CD4 T lymphocytes.

Monokines

Monocytes of blood and tissue macrophages promote the development and differentiation of T and B lymphocytes, and hematopoietic cells. Their lymphokines are named “monokines.

Lymphokines

Some interleukins are classified as lymphokines, lymphocyte-produced cytokines that mediate immune responses.

Major Interleukins, Cytokines & their functions

Interleukin/ cytokine	Source cells	Target cells	Biological activity
IL-1	Macrophages, monocytes & other cells.	T & B lymphocytes.	Pro-inflammatory; Stimulates T cells; Pyrogen.
IL-2	TH0, TH1 lymphocytes.	T & B lymphocytes.	Activates T cells and B cells.
IL-3	T lymphocytes, mast cells.	Hematopoietic stem cells.	Hemopoietic growth factor.
IL-4	TH2 lymphocytes macrophages.	B lymphocytes, TH1 lymphocytes, macrophages.	B cell Growth & differentiation factor.
IL-5	TH2 lymphocytes Macrophages.	Eosinophils, lymphocytes.	Chemotactic & activating factor for eosinophils & Lymphocyte.
IL-6	B & T lymphocytes Macrophages.	B & T lymphocytes & other cells.	B-cell differentiation factor.
IL-7	Bone marrow, thymic epithelium.	B & T Pro-lymphocytes.	Lymphoid cell growth factor.
IL-8	Macrophages.	Neutrophils, T lymphocytes.	Neutrophil and T-lymphocyte chemotactic factor.

https://www.researchgate.net/figure/Cytokines-produced-by-several-types-of-immune-cells_tbl1_312151668

Figure 3. Activity of cytokines

There are interleukins, interferons, tumor necrosis factor alpha (TNF-a), and transforming growth factor beta (TGF-b) are shown on the pictures.

2.6. ANTIGENS OF HUMAN HISTOCOMPATIBILITY AND THEIR VALUE IN THE IMMUNE RESPONSE

Direct specific interaction of immune cells

There are some tolls of immune system where the specific interaction between immune cells with specialized surface structure and receptors to it or on all nucleotide cells has place. There are histocompatibility antigens - glycoprotein molecules on all

nucleotide cells. It is named “Major histocompatibility complex antigens (MHC)” and “Human leucocyte antigen (HLA)”.

Major Histocompatibility Complex Antigens (MHC) has an important function in presentation of antigens to T-cells. CD4 T cells recognize foreign antigens on surface of APCs (macrophage), only when these antigens are presented in the groove of MHC II molecule. Cytotoxic T-cells will recognize antigens, on the surfaces of virus infected cells or tumor cells only when these antigens are presented in the groove of Class I molecule (MHC restriction).

Such interaction is very specific, but it is not direct fight with something foreign. It is part of effective mechanism for fight with some another really foreign. So, in this context term “antigen” has not the same meaning like for foreign substances.

HLA (human leucocytes antigen) complex

In humans, the HLA complex of genes is located on short arm of chromosome 6 containing several genes that are critical to immune function. The HLA complex of genes is classified into three classes as follows:

1. **Class I: HLA-A, HLA-B, and HLA-C.**
2. **Class II: HLA-DR, HLA-DQ, and HLA-DP**. All of these are present within HLA-D region of HLA complex.
3. **Class III**: Complement loci that encode for C2, C4, and factor B of complement system and TNFs alpha and beta.

Gene products of HLA complex

In human, like another vertebrate, there is a genetic region that has a major influence on graft survival. This region is referred to as the Major Histocompatibility Complex (MHC). Individuals identical for this region can exchange grafts more successfully than MHC non-identical combinations. The MHC antigens play an important role in antigen recognition by T cells.

Class I MHC genes encode glycoproteins expressed on the surface of nearly all nucleated cells; the major function of the class I gene products is presentation of endogenous peptide antigens to CD8T cells (T cytotoxic).

Class II MHC genes encode glycoproteins expressed predominantly on APCs (macrophages, dendritic cells, and B cells), where they primarily present exogenous antigenic peptides to CD4T cells (CD4 T cells T).

Class III MHC genes encode several different proteins, some with immune functions, including components of the complement system and molecules involved in inflammation.

MHC I antigen

The largest part of the heavy chain of the MHC I antigen is organized into three globular domains (α1, α2 and α3) which protrude from the cell surface. The heavy chain has a variable and constant region.

The variable region is highly pleomorphic. The polymorphism of these molecules is important in the recognition of self and non-self. The peptide-binding groove is located on the top surface of the class I MHC molecule, and bind a peptide of 8 to 10 amino acids.

The constant region of the heavy chain binds with the CD8 proteins of the cytotoxic T cells.

MHC II antigen

Class II MHC molecules are also transmembrane glycoproteins, consisting of α and β polypeptide chains. The α2 and β2 domains, the ones nearest to the cell membrane, while the α1 and β1 domains form the peptide-binding groove for processed antigen.

Class II proteins are primarily responsible for the graft-versus-host response and the mixed leukocyte response.

Processing and presentation of an antigen by antigenpresenting cell

Direct specific interaction of the immune cells is necessary to transmit the information about foreign antigenic substance. Induction of immune response begins when an antigen penetrates epithelial surfaces. It will eventually come into contact with macrophages or certain other classes of Antigen Presenting cells (APCs), which include monocytes, dendritic cells, Langerhans cells, endothelial cells and B-cells.

Antigens, such as bacterial cells, are internalized by endocytosis and "processed" by the APC, then "presented" to immunocompetent lymphocytes to initiate the early steps of the specific immune response.

Processing by a macrophage (for example) results in the selective destruction of foreign substance with preservation of antigenically significant part (antigenic determinant), attaching of the processed antigenic structure to the surface of the cell in association with MHC II molecules of the APCs.

To recognize antigen, T-cells have special receptors - T-Cell Receptor (TCR) only recognizes antigen presented by MHC molecules on another cell, the Antigen Presenting Cell. The TCR is specific for antigen (like antibodies), but the antigen must be presented on a self-MHC molecule.

The TCR is also specific for the MHC molecule. If the antigen is presented by another allelic form of the MHC molecule in vitro (usually in an experimental

situation), there is no recognition by the TCR. This phenomenon is known as MHC restriction.

The antigen+class II MHC complex is presented to a CD4 T cells (Th2), which is able to recognize processed antigen in the antigen+class II MHC complex on the membrane of the macrophage by MHC II receptor, which is closely linked together on its membrane. So, specific information about structure of the foreign antigen is transmitted directly from own cell to own cell. It is named as **dual recognition process.**

The interaction of the macrophage and Th2- CD4 T cells, together with stimulation by Interleukin 1 (IL-1), produced by the macrophage, activates the Th2-cell. Activation of the Th2-cell causes that cell to begin to produce Interleukin 2 (IL-2), and to express a membrane receptor for IL-2. The secreted IL-2 autostimulates proliferation of the Th2-cells. Stimulated Th2-cells produce a variety of lymphokines including IL-2, IL-4, IL-6, and gamma Interferon, which mediate various aspects of the immune response.

For example, IL-2 binds to IL-2 receptors on other T-cells (which have bound the Ag) and stimulates their proliferation, while IL-4 causes B-cells to proliferate and differentiate into antibody-secreting plasma cells and memory B-cells. The overall B-cell response leads to antibody-mediated immunity (AMI).

Activation of B cells

Specific receptor of B cells for the antigenic determinant is antibody-receptor (Ig receptor). And it is supported by MHC complex+MHC receptor interaction too. For the interaction of CD4 T cells and B cell additionaly to the dual recognition, the direct interaction of **CD4 T cells CD40L marker (antigen) and B cell CD40 receptor is nesessery**.

Plasma cells are relatively short-lived (about one week) but produce large amounts of antibody during this period. Memory cells, on the other hand, are relatively long-lived and upon subsequent exposure to Ag they become quickly transformed into Ab-producing plasma cells.

B lymphocytes are capable to receive free, soluble antigen without macrophages and CD4 T cells.

Activation of cell mediated immunity

Generation of cell-mediated immunity (CMI), like humoral immunity, begins when a T-cytotoxic cell (TC) recognizes a processed antigen associated with MHC II molecules on a CD4 T cells. It is dual recognition process, like in cause of the humoral immunity. But TC have another type of the specific cellular receptor for foreign antigen. It is a special T-cell receptor.

T-cytotoxic cell can also recognize antigen directly, without CD4 T cells, when it is connected with MHC I on the membrane of a tissue cell, not immune cell (usually an altered self-cell, but possibly a transplanted tissue cell or a eukaryotic parasite). Under stimulation by IL-2 produced by Th2-cells the TC-cell becomes activated to mature to a cytotoxic T-lymphocyte (CTL, CD8 cytotoxic T), capable of lysis the cell, which is showing the new (foreign) antigen on its surface (a primary manifestation of CMI).

So, peptide antigens associated with class I MHC molecules are recognized by CD8 cytotoxic T lymphocytes, whereas class II-associated peptide antigens are recognized by CD4 T cells.

2.7. ANTIBODIES AS PRODUCT OF HUMORAL IMMUNE RESPONSE. STRUCTURE AND FUNCTION OF ANTIBODIES (IMMUNOGLOBULINS). THE CONCEPT OF THE VALENCE OF ANTIBODIES

Antibodies, also called immunoglobulins, are proteins manufactured by the body that help fight against foreign substances called antigens. When an antigen enters the body, it stimulates the immune system to produce antibodies (the immune system is the body's natural defence system). The antibodies attach, or bind to the antigen and inactivate it.

Antibodies (immunoglobulins, Ig) are substances produced by mature B cells (plasma cells) in response to an antigenic sign, serve as a protective agent against antigens.

The term “antibodies” refers to a group of related proteins which are capable of specific, noncovalent binding to the molecules which induce their manufacturing. Antibody molecules are typically Y-shaped, with a binding site on each arm of the Y.

These are globular structure proteins, gamma-fraction of the serum proteins. They are formed in response to antigenic stimulation and react with corresponding antigen in a specific and observable manner. The antibodies are factors of adaptive immunity. And it is humoral immunity because of solubility of antibodies in the host’s body liquids.

The molecule consists of two heavy polypeptide chains and two light polypeptide chains, held together by disulfide bridges as shown. The molecule can be hydrolyzed with papain to produce three fragments: two identical fragments, called fragment antigen-binding (Fab), each of which contains one antigen-binding site, and fragment crystallizable (Fc), which contains sites that determine the binding of antibody to specific host cells. Different molecules of antibodies are specific for

interaction with different antigens (different size, form). It's why Fab has variable structure. It is variable part of an Ig or **paratop**.

2.8. CLASSES OF IMMUNOGLOBULINS, THEIR STRUCTURE AND PROPERTIES

Antibodies classification: five classes of immunoglobulins have been distinguished: IgG, IgA, IgM, IgD and IgE.

It was decided that structure which is like to the IgG is monomer for any antibody. Monomer of Ig has one Fc and two Fab. Monomeric structure is typical for IgG, IgE and IgD. IgA is dimer (two monomers together) and IgM is pentamer (five monomers together).

The classes of antibodies are different in location in a body and in function.

The main immunoglobulins working in blood are IgM and IgG. IgM is produced first after antigen invasion. Then the immune response is switched to IgG production. The IgG is the mainefor mature immune response. That class of Ig has the biggest opsonization and neutralization activities.

IgA is the main in mucosal tissues and it is secreted with tears, saliva, milk, intestinal mucosa secretes. It is monomer in the blood and dimer or even trimer in tissues. Transfering with milk from mother to child it creates the passive adaptive immune defence of the mucus membranes for the child.

The mostly possible function of the IgD is function of the antibody receptor on the B cell.

IgE is involved in allergy (immediate, type I – anaphylactic type). It is bound to mast and basophil cells throughout the body and present in blood.

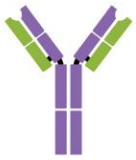
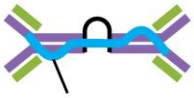
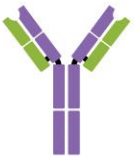
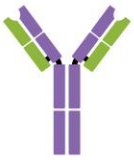
The biggest part is for the IgG (80%), next is for IgM (5-10%). IgA is present with concentration of 10 to 15%, ad IgD – 0,2%. The smallest concentration is for IgD – 0,002% only.

Because of immune system (named such by function for a body) is the lymphatic system structurally and the lymphatic system produces these substances, all the classes of immunoglobulins are present in the lymph.

Complement fixation function of antibodies is important because it is the moment complement system activation by classical pathway. This pathway is the mostly effective for activation of this humoral nonspecific cascade mechanism. Such complement fixating possibility is typical for IgG and IgM only.

Placental transfer is the possibility for IgG only. It is way for child to get effective adaptive immunity before birth. It is passive immunity because of the ready antibody molecules transfer from mother to child.

Antibodies valence is determined by the number of active centres (**Fab** fragments). Divalent (IgG) and ten-valent (IgM) immunoglobulins are complete antibodies. In the interaction of antigens with IgG and IgM, giant immune complexes are formed, which can be visually detected in certain serological reactions.

The Five Immunoglobulin (Ig) Classes					
Properties	**IgG monomer**	**IgM pentamer**	**Secretory IgA dimer**	**IgD monomer**	**IgE monomer**
Structure			 Secretory component		
Heavy chains	γ	μ	α	δ	ε
Number of antigen-binding sites	2	10	4	2	2
Molecular weight (Daltons)	150,000	900,000	385,000	180,000	200,000
Percentage of total antibody in serum	80%	6%	13% (monomer)	<1%	<1%
Crosses placenta	yes	no	no	no	no
Fixes complement	yes	yes	no	no	no
Fc binds to	phagocytes				mast cells and basophils
Function	Neutralization, agglutination, complement activation, opsonization, and antibody-dependent cell-mediated cytotoxicity.	Neutralization, agglutination, and complement activation. The monomer form serves as the B-cell receptor.	Neutralization and trapping of pathogens in mucus.	B-cell receptor.	Activation of basophils and mast cells against parasites and allergens.

https://openstax.org/books/microbiology/pages/18-1-overview-of-specific-adaptive-immunity

Figure 4. Classes of the immunoglobulins: properties of immunoglobulins

Monovalent immunoglobulins with one Fab (incomplete, blocking antibodies) sometimes are present. These are not able to form immune complexes with antigens. The results of this interaction can not be visually detected in serological reactions. The presence of incomplete antibodies is detected by the Coombs antiglobulin test.

2.9. ANTIGENIC STRUCTURE OF IMMUNOGLOBULINS: ISO-, ALO-, IDIOTYPIC DETERMINANTS. PRACTICAL USE

Antigenic structure of immunoglobulins

Immunoglobulins consist of proteins it is why when transferred to another organism these are antigens. It distinguished some different types of the immunoglobulin antigens (types, isomers).

Isotypes (isomers) are characterized the affiliation of an immunoglobulin to a certain class **(M, G, A, E, D)**; are species-specific and antigenic difference is present in C-region (Fc).

Allotypes (because of allelic genes for that are present) - alter variants of immunoglobulin within classes and subclasses; are individual. Antigenic feature of Ig that vary among individual under genetic control Ag difference in C-region of H and L chain.

Idiotypes are variants of immunoglobulins, which differ in the number and order of alternation of amino acids in the Ig active center (Fab, variable). This pattern of Fab for every antigen is different even in the same host. Idiotype is the same even in antibodies from different hosts, if it is for the same antigen. Just about that they say if the specific antigen and antibody compliance are compared with the key and the lock compliance. Only antigens that match this shape will fit into them. After understanding that, it is possible to realize, that it is possible to get specific anti-antibodies and that even can be used instead of real antigen, in spite of first one is pathogen and last one is antibody.

Practical use of this theoretical knowledge is necessary in blood transfusion, transplantation, specific prevention and treatment of infectious diseases.

2.10. HUMORAL IMMUNE RESPONSE AND ITS STAGES. PRIMARY AND SECONDARY IMMUNE RESPONSE

Dynamic of antibodies formation

The humoral response (or antibody mediated response) involves B cells that recognize antigens or pathogens that are circulating in the lymph or blood (“humor” is a medieval term for body fluid).

With the humoral immune response, the effector is the descendants of B-lymphocytes - plasma cells, or more precisely the products of their vital activity – antibodies.

Antibodies acquire in the embryonic period of the macroorganism from the 13th week and IgM can be detected in serum from the 20th week. The process of the

continuous appearance of new antibodies producing cells of different specificity which form the baseline basal level of antibodies (mainly the isotype M - normal antibodies) begins from this moment. In the post-embryonal period the content of immunoglobulins in the serum may be vary substantially depending on the age and the state of the macroorganism. Normal antibodies are constantly formed in the body without antigenic stimulation. Their presence indicates the readiness of the macroorganism for immune response, as well as the possible remote contact with antigen.

The specificity of antibodies is one only for every separate B cell. It is determined by the cell surface antibody receptor's specificity. The specificity is predetermined on the antigen independent stage of every cell development in the bone marrow.

Distinguish forms of immune response are:

1) the primary immune response (at the first encounter with antigen);
2) the secondary immune response (at a repeated meeting with an antigen).

The dynamics of antibody formation essentially depends on the primary or secondary contact of the immune system of the macroorganism with the antigen. The dynamics and intensity of antibody formation depends on a large extent of the immunogenicity of the antigen (dose, method and type of administration) and also on the state of the macroorganism.

The primary immune response

The biosynthesis of specific antibodies is amplified with the appearance of the antigen in the internal environment (in the body). First interaction of the surface B-cell antibody receptor with free soluble antigen, or with presented one by CD4 T cells in connection with MHC II of the CD4 T cells (antigen dependent stage) is the specific sign for proliferation of the B-cell (clone-formation) and then maturation of the B-cell till plasma cells (antibody producers). It is the essence of the primary immune response. The B lymphocytes are responsible for secreting Ig antibodies and can also function as highly efficient antigen-presenting cells (APCs) for T lymphocytes.

Stages of the primary immune responce

Allocate the next stages of the primary immune responce:

1) the first one - inductive (presentation and recognition of the antigen, interaction of immunocompetent cells). Antigens bind to B cells.

Interleukins 2 produced by CD4 T cells costimulate B cells. In most cases, both an antigen and a costimulator are required to activate a B cell and initiate B cell proliferation;

2) the second - productive (proliferation of effector cells, maturation B-cells to plasma cells and the antibody production). B cells proliferate and produce plasma cells. The plasma cells bear antibodies with the identical antigen specificity as the antigen receptors of the activated B cells. The antibodies are released and circulate through the body, binding to antigens;

3) **stationary phase**;

4) **phase of decrease**.

In this case, the intensity of the primary immune response reaches maximum within 7-8 days, persists for 2 weeks, and then decreases.

Characteristics of immune response

The immune response is characterized by:

1. **specificity** (reactivity is directed only at a certain agent, which is called an antigen, antibodie's paratop interacts with own epitope of antigen only);
2. **potentiation** (the ability to produce an enhanced response with a constant intake of the same antigen into the body);
3. **immunological memory** (the ability to recognize and produce an enhanced response against the same antigen upon repeated exposure to the body, even if the first and subsequent hits occur at large intervals). B cells produce memory cells. Memory cells provide future secondary immune responses. T- and B-lymphocytes memory cells are carriers of immunological memory.

Immunological memory

After first exposure to pathogen and its recognition it is 3 to 6 days lag faze and then antibodies peak in 10 to 12 days. Later the B, CD4 T cells and T cytotoxic memory cells are created. It is the basis for immunity from the disease.

The secondary immune response

Adaptive immunity keeps the memory. The secondary immune response develops faster and reaches greater (3-4 times) intensity. For that memory, B and T cells immediately identify the pathogen and act faster, longer, more effective then for first response. At subsequent B and T cells clones form in a few days. Often no symptoms of the disease are noticed.

Major characteristics/differences between primary and secondary immune response is summarized in this table:

Table 10. Major characteristics/differences between primary and secondary immune response

Characteristics	Primary Immune Response	Secondary Immune Response
Definition	Immune response against primary antigenic challenge	Immune response against subsequent antigenic challenge
Response	Low, sluggish (appear late) and short lived	Prompt, powerful and prolonged (long lasting)
Antibody producing cells	Naïve B cells	Memory B cells
Peak Response	Smaller	Larger
Antibody levels	Antibody levels peak in the primary response at about day 14 and then begin to drop off as the plasma cells begin to die.	Because there are many more memory cells than there were naïve B cells for the primary response, more plasma cells are generated in the secondary response, and antibody levels are consequently 100 to 1000 fold higher.
Lag period	Lag period is longer (4-7 days) This lag is the time required for activation of naive B cells by antigen and TH cells and for the subsequent proliferation and differentiation of the activated B cells into plasma cells.	Lag period is absent or short (1-3 days) The secondary response reflects the activity of the clonally expanded population of memory B cells. These memory B cells respond to antigen more rapidly than naïve B cells.
Negative phase	No negative phase	Negative phase may occur
Antibody Isotype	Antibody produced in low titer and is of IgM type. In the disease diagnosis presence	Antibody produced is high titer and mainly is of IgG type (IgA or IgE in certain situations).

	of IgM is suggestive of recent primary infection.	In the diagnostics, presence of IgG should be interpreted cautiously as it may be because of previous vaccination, subsequent sub-clinical infections (local cut off titre or previous infections with the same agent).
Specificity of Antibody	Antibodies are more specific but less avid	Antibodies are less specific but more avid
Antibody Affinity	Lower average affinity, more variable	Higher average affinity (affinity maturation)
Induced by	All immunogens (Both T dependent and T independent antigens are processed by primary immune response)	Only protein antigens (Only T dependent antigens are processed)

https://microbeonline.com/differences-between-primary-secondary-immune-response/immunological tolerance.

Immunological tolerance

Immunological tolerance is the phenomenon opposite of memory - in response to the reintroduction of the antigen, the body, instead of vigorously rapid formation of immunity, shows irreactivity, does not correspond to the immune response, that is, tolerant (patient) to the antigen introduced. The immunological tolerance dose of an antigen can be both – bigger and smaller then immune doze and is different for different antigens.

2.11. CELLULAR IMMUNE RESPONSE AND ITS STAGES

Principles of the cellular adaptive immune response formation and its stages

The cell mediated response involves mostly T cells and responds to any cell that displays aberrant antigens in specific connection with MHC markers, including cells invaded by pathogens (with intracellular parasite like virus), tumor cells, or transplanted cells.

Principles of the cellular adaptive immune response formation and its stages are very similar to the same for humoral immunity.

The mostly significant differences are in the next positions:

1. T-cytotoxic cells (TCT, CD8 effector cells, or T8+ cells) and Normal killers are effector cells in this case. (To a lesser extent – CD4 cells).;
2. Specific cellular T-cell receptors for antigens are different from antibody receptors of B-cells;
3. Extracellular killing is the mechanism to attack the target cells;
4. Difference in costimulation of immune response by cytokins.

It should be born in mind that CD4 cells are also completely specific to certain antigens, they are part of the specific immunity. In addition, they can play not only a regulatory role (as the main), but also synthesize a number of substances to perform effector function (effector cytokines).

CD4 T cells are divided into a number of subpopulations that differ in the range of functions performed (Th1, Th2, Th17, T regs).

The following chain of events describes this immune response:

1. Self-nonimmune cells or APCs displaying foreign antigens bind to T cells;
2. Interleukins (secreted by APCs or CD4 T cells) costimulate activation of T cells;
3. If MHC II and exogenous antigens are displayed on the plasma membrane of APCs, T cells proliferate, producing CD4 T cells and cytotoxic T cells specific clones;
4. CD4 T cells display foreign antigens bind to B cells. B cells proliferate, producing appropriate specific clones;
5. CD4 T cells release interleukins (and other cytokines), which stimulate B cells to produce antibodies that bind to the antigens, and stimulate activity of nonspecific agents (NK and macrophages) to destroy the antigens;
6. If MHC I and endogenous antigens are displayed on the plasma membrane self nonimmune cells, T cells proliferate, producing cytotoxic T cells;
7. Cytotoxic T cells destroy cells displaying the antigens.

Mechanism of cell killing by activated cytotoxic CD8 T cell

Cytotoxic CD8 T cells, when connect a target cells whis **specific TCR**, release biologically active proteins – **perforins**. The proteins (perforin, granzyme B, and Fas ligand) are available to kill the target cell. Perforin causes fenestrations (windows) within the cell membrane, allowing entry of granzyme B and Fas ligand.

Cytotoxic T cells have also nonspecific **Fas ligand (or Apo-1, CD95 ligand)** for interaction with Fas (or death receptor) on serface membrane of the host cell.

Granzyme B and Fas ligand induce apoptosis via two different mechanisms leading to target cell death.

The apoptosis is especial mechanism of programmed cell deth.

This mechanism of immune defence is effective to tomor cells and virus infected cells. Transplanted cells (**grafts**), as foreign, are recognized by T cytotoxic cells too.

Normal killer (NK) cells

NK cells have the ability to kill target cells **without activation** by antigens displayed by antigen-presenting cells, and **without prior training**, unlike other immune cells that have to learn to recognise a target. As T cells require MHC for activation, it has led to some viruses and cancer cells evolving to remove MHC molecules from the surface of infected cells to avoid detection. NK cells are the cells that lack these surface markers.

NK cells are not governed by single receptors but regulated by the integration of co-activating (NKG2D, NKp46 and LFA-I) and co-inhibitory (including NKG2A, TIGIT and KLRG1) signals via cell surface receptors that recognise their corresponding ligands on healthy cells or target cells to determine the responsiveness of the NK cells.

NK cells use two main mechanisms to respond to inflamed tissues or damaged cells:

1. Firstly, **by detecting the presence of stress signals released by infected or tumor cells**. This is thought to be a type of ‘self-sacrifice’ (**apoptosis**). The activating and inhibitory receptor signalling regulates the natural killer (NK) cells activation. Cells undergoing stress such as tumor cells lose their MHC class I molecules, a ligand for inhibitory receptors on NK cells. At the same time, they acquire stress-associated molecules which act as ligands for activating receptors. Thus, the lack of inhibitory signalling coupled with induction of activating signalling shifts the balance toward NK cell activation, leading to secretion of cytokines and killing of target cells;
2. The second mechanism is **the indirect activation by specific antibody Fc-receptor** on the surface of target cell.

NK cells **kill the target cells by releasing cytotoxic granules** containing perforin and granzymes.

2.12. CHARACTERISTICS OF MANIFESTATIONS OF IMMUNE RESPONSE

Innate immunity manifestation

Many humoral and cellular factors of innate response are present in the host. Everyone has its own mechanism of defence and it has described **in the Part 1**.

Pathogens include bacteria, viruses, and other organisms, which cause infections, can cause **inflammation**. It is complex result of activity of many humoral and cellular factors of innate defence, which are active together at the same time. If it is **local inflammation,** it manifestetes by **Pain, Redness, Loss of function, Swelling and Heat.** It is because of many humoral and cellular factors come to the damaged location and are active there. It creates the optimal condition for effective fight with foreign substance, but at the same time some negativeness for host.

Humoral adaptive immunity manifestation

Triggering feedback innate defence reaction mechanisms

These complexes antigen-antibody are insoluble particles and can therefore be disposed by phagocytes.

To be connected with antigen by Fab fragment, interact with another cells and substances by Fc fragment. This interaction:

1. help with phagocytosis (opsonization);
2. activate the complement system (classical pathway)
3. activates of the NK-cells, if foreign antigen is recognized by antibody's Fab on a surface of the host cell and the target cell is destroyed by the NK cell activity;
4. leads to degranulation of mast cells and release of mediators of inflammation - histamine and serotonin.

Effects at the specific level:

1. Neutralization of viruses by connection with viral surface receptors to specific cellular surface structures;
2. Neutralization of bacterial protein toxins by connection with its active centres;
3. Neutralization of bacterial surface receptors to specific cellular surface structures. After that, infection of the cell is impossible.

Cellular adaptive immunity manifestation

T cytotoxic cells are effector cells for this part of the immune defence. The target for it always is host's cell with changed properties. It can be infected cell, or

tumor cell, or false hosts (transplanted). These targets are destroyed extracellularly by apoptosis.

2.13. IMMUNOPATHOLOGY

Innate factors

Inflammation plays positive role in innate immune response, but also leads to tissue damage. Severe adverse effects cause chronic inflammation. Worldwide, 3 of 5 people die due to chronic inflammatory diseases like stroke, chronic respiratory diseases, heart disorders, cancer, obesity, and diabetes.

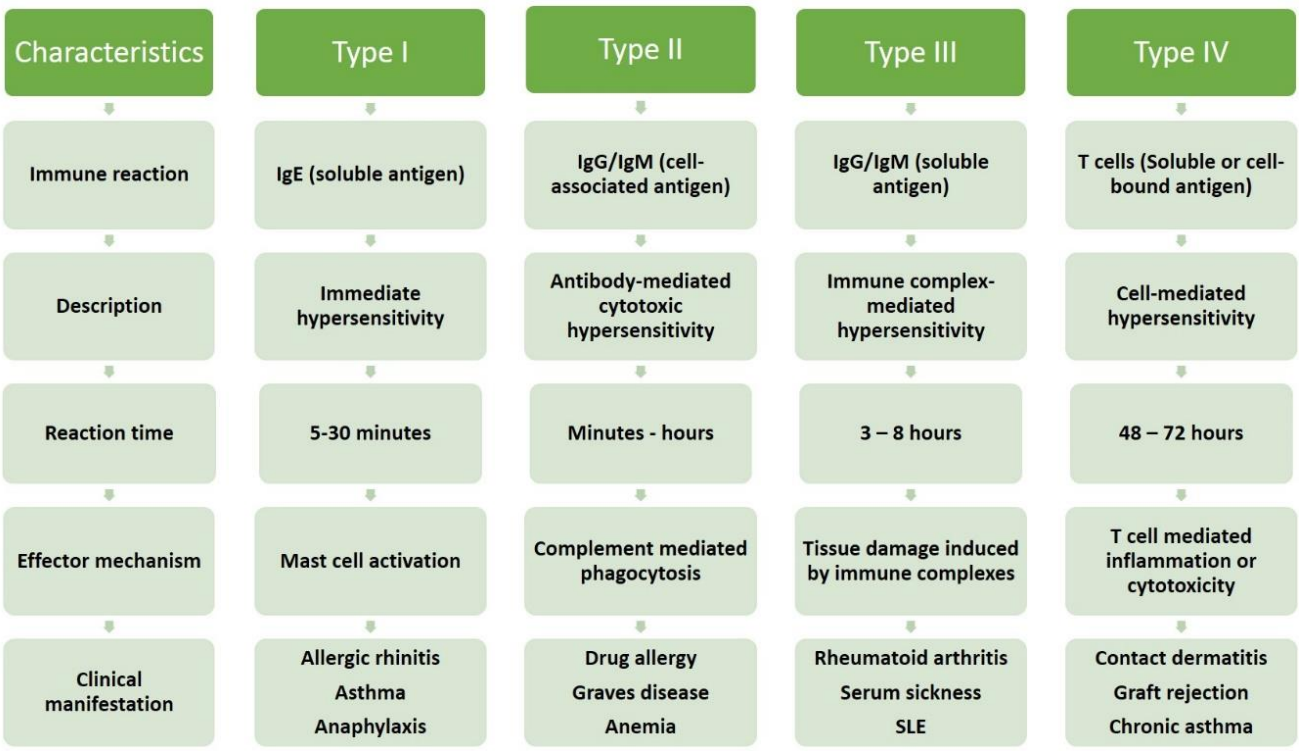
Blood coagulation and inflammation are universal responses to infection and there is crosstalk between inflammation and coagulation that can either amplify or dampen the responses. Loss of appropriate interactions between these systems probably contributes to morbidity and mortality in infectious diseases. For instance, **inflammatory cytokines and leukocyte elastase** can downregulate natural anticoagulant proteins that help to maintain endothelial-cell integrity, control clotting, inhibit vasoactive peptides and dampen leukocyte infiltration into the vessel wall.

A **cytokine storm** is an overreaction of the body's immune system. . It consists of a positive feedback loop between cytokines and immune cells and can lead to death.

Allergic reactions (hypersensitivity)

Allergies are caused by undesirable immunological reactions produced by the host’s immune system to substances that are commonly considered harmless.

Allergic reactions are categorized into four types of hypersensitivity based on the underlying mechanism. **Type I, II and III are antibody mediated while type IV is cell-mediated.**



http://gbsleiden.com/allergy/

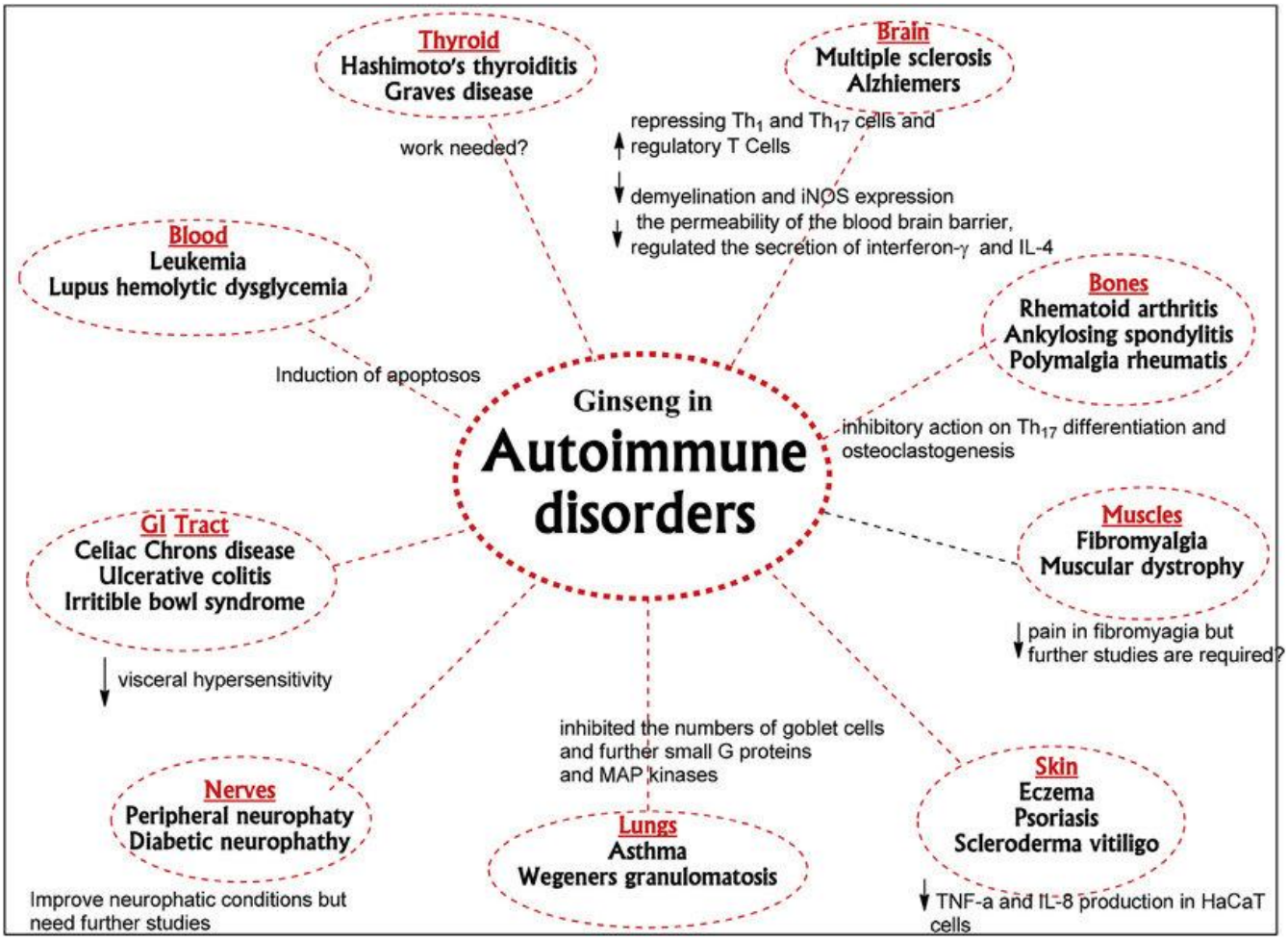
Figure 5. Types of allergic reactions

Autoimmune diseases

An **autoimmune disease** is arising from an abnormal immune response to a "normal" body structures. There are more than 80 types of autoimmune diseases. The cause of the diseases is generally unknown. Sometimes it is triggered by infection, or by environmental factor, or heredity.

These diseases tend to have characteristic pathological effects, which characterize them as an autoimmune disease. Such features include damage to or destruction of tissues where there is an abnormal immune response, altered organ growth, and altered organ function depending on the location of the disease.

Autoimmune diseases present similar symptoms. The appearance and severity of these signs and symptoms depends on the location and type of autoimmune response that occurs. Some diseases are organ specific and are restricted to affecting certain tissues. There are several areas that are commonly impacted by autoimmune diseases. These areas include **blood vessels, underlying connective tissues, joints and muscles, red blood cells, skin, and endocrine glands, like thyroid or pancreas glands.** Others diseases are systemic that influence many tissues throughout the body.



https://www.researchgate.net/figure/Ginseng-and-autoimmune-disorders-Ginseng-or-its-preparations-have-been-reported-to-have_fig3_328833463

Figure 6. Examples of the autoimmune diseases

PART 3. SEROLOGICAL REACTIONS

3.1. SEROLOGICAL REACTIONS. MECHANISM OF INTERACTION OF ANTIGENS AND ANTIBODIES IN SEROLOGICAL REACTIONS. THE MAIN COMPONENTS OF SEROLOGICAL REACTIONS

Serological reactions

The serological reactions are the reactions in vitro between antigen and antibody. This is called “serological” because the blood serum with antibodies is used here.

The main components of serological reactions

Antigen and antibody are the main components of serological reactions. For visible reaction in vitro it is important to make reaction with equivalent ratio of the components. In this case only it is created the lattice of antigen-antibody connections that increases size of the particles to visible level.

Features of antigen-antibody interaction reactions:

1. The reaction is highly specific.
2. There is no denaturation of antigen or antibody during reactions.
3. Combination occurs at surface and hence surface antigens are immunologically relevant.
4. The combination is firm and it is influenced by affinity and avidity.
5. Affinity is intensity of attraction between antigen and antibody molecules.
6. Avidity is strength of the bond after the formation of antigen antibody complex.
7. Both antigen and antibody participate in the formation of the results agglutinates and precipitates.
8. Antigen and antibody may combine in varying proportions.

3.2. PRACTICAL USE OF SEROLOGICAL REACTIONS: IDENTIFICATION OF ANTIGEN, DIAGNOSTIC DETECTION OF ANTIBODIES. DIAGNOSTIC IMMUNE SERUMS, DIAGNOSTIC BACTERIAL SUSPENSION

On the base of known component we can detect unknown one and put correct conclusion about diagnosis. Antigen-antibody reactions are useful in laboratory

diagnosis of various diseases and in the identification of infectious agents in epidemiological aim.

Serological reactions can be used in the different directions (methods) of the microbiological diagnostics of the infections diseases:

In the **cultural (bacteriological or virological)** for determining of the pathogen's antigenic structure. It is important for identification of the pathogen;

In the **biological**, when final result of the serological reaction can be revealed after injection of the antigens and antibodies mixture in a laboratory animal's organism.

Sometimes it is possible to **direct (express) revealing** of pathogen in serological reaction.

In the **serological method** when the purpose is to reveal fact of immune response formation to the pathogen by revealing of the specific antibodies to the pathogen in the serum of the patient.

Diagnostic immune serums

Diagnostic immune serum (standard serum) is the immunobiological preparation that contains specific (known) antibodies to the pathogenic microorganisms, another unknown antigens.

Diagnostic bacterial suspension (standard antigens)

Diagnosticums (standard antigens) are the immunobiological preparations that contain a known antigen of or from the pathogenic microorganisms, another important substances.

3.3. REACTIONS BASED ON THE PHENOMENON OF AGGLUTINATION: DIRECT AND INDIRECT AGGLUTINATION, REVERSE HEMAGGLUTINATION TEST. PRACTICAL USE

Agglutination tests

Agglutination phenomenon is based on interaction between antibodies (agglutinins) and antigens (**agglutinogens**), located on the surface of a bacterial cell, when a particle antigen (insoluble, bacterial cells) is mixed with its antibody in presence of electrolytes at a suitable temperature and pH, then the particles are clumped or agglutinated.

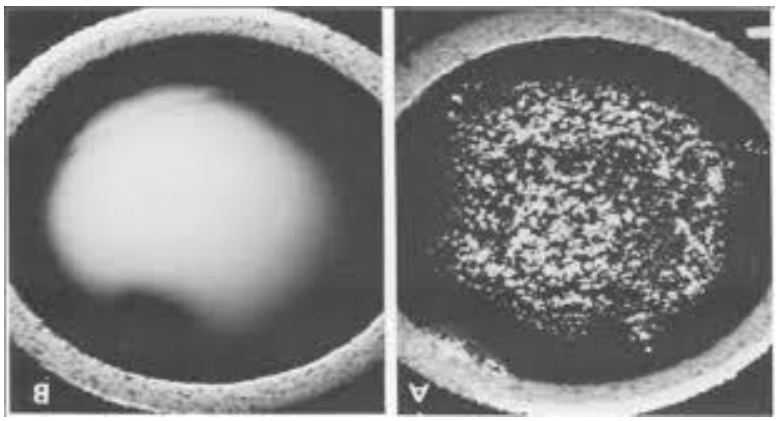
Slide agglutination

Slide agglutination is carried out on a clean slide by mixing of standard antiserum and investigated antigen – bacterial cells from an isolated colony or from pure culture **(fig. 7).**

Materials for test are:

• Culture of bacteria;
• Adsorbed immune diagnostic serum (IDS) for glass-agglutination test;
• 0,5% solution of NaCl;
• Glass for smears, bacteriological loop.

Reaction occurs immediately. Positive test is manifested by clumping formation. It is used in the bacteriological method for bacterial culture identifying.



https://www.slideserve.com/leone/antigen-antibody-reactions

Figure 7. Slide agglutination test

Types of agglutination observed with bacterial antigen

Flagella antigen or H-type of agglutination is seen when a formalized suspension of motile bacteria in treated with antiserum. It forms snowflakes deposit. Agglutination appears from 2 to 4 hours after incubation at 52^{o}C.

Somatic O-type agglutination is compact with fine granulation. The reaction appears from 18 to 24 hours after incubation at 37^{o}C.

Vi-agglutination (Vi-antigen is microcapsule antigen) is similar to O-agglutination and occurs slowly at 37^{o}C.

Tube agglutination

Tube agglutination is carried out as a quantitative test to estimate the titter of antibody in a serum or to confirm the result of slide agglutination. It can be important to estimate level of the immunity. It cannot be done easily with estimation of the antibody concentration by biochemical technique, it's why has place this technique of the indirect evaluation by serial dilution of a serum and estimation of the effective

dilution. **Titter of the tube agglutination test is the maximal dilution with positive result.**

Patient's blood sera must be dissolved by isotonic solution (saline solution) in tubes for this case according to table:

Table 11. Scheme of agglutination test for finding antibodies in patient serum

Ingredient (in ml)	Number of the test tube						
	1	2	3	4	5	6	7 serum control
Saline solution	1,0	1,0	1,0	1,0	1,0	1,0	-
The patient's serum in a 1:10 dilution (0,1 ml serum + 0,9 ml isotonic saline solution)	1,0	1,0	1,0	1,0	1,0	1,0	1,0
Standard antigen	0,1	0,1	0,1	0,1	0,1	0,1	-
The obtained dilution of the serum	1:20	1:40	1:80	1:160	1:320	1:640	1:10

The previous results of reaction are registered after 2 hours, and final – after 18-20 hours of thermostat incubation at 37^{o}C.

Titter of the reaction is 1:160 *(figure 8).* To understand is it big or small we should compare it with something known earlier. It should be compared with so named diagnostic titter.

Diagnostic titter is the maximal dilution of the sera that demonstrates the specific immune response to supposed pathogen. If real titter is equal or bigger then diagnostic titter, it should be done conclusion about the real infection disease is equal to supposed one. For every disease and every serological reaction diagnostic titters are not connected and can be different.

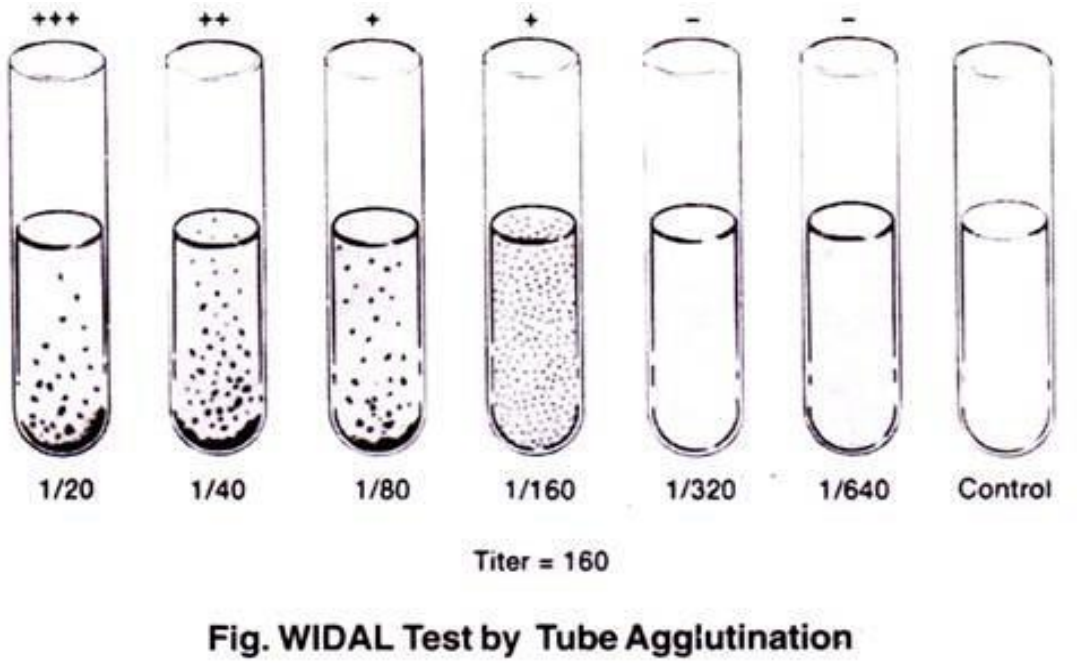


Fig. WIDAL Test by Tube Agglutination

http://www.biologydiscussion.com/bacteria/7-main-serological-tests-to-prevent-bacterial-disease-microbiology/85828

Figure 8. Tube agglutination test

Pared sera technique

Sometimes the way of the comparing standard and experimental titters are not working because some infectious diseases don't give strong immunity to distinguish positive and negative reactions when initial level of the "normal" titter is different for different people because they have different immune status (the power of immunity at repose).

To confirm the reaction is positive in this case it should be used **pared sera technique** when the comparing between two sera of the same patient has place. These sera are different in period of the disease when it was taken. First one is taken in first days of the disease ("zero" level of the titter), second one is taken from 7th to 14th day – in one – two weeks of disease), when the titter is increased because of the stages of immune response formation. **If the increase of the titter in 4 or more times is revealed, it means reaction is positive.**

Indirect (passive) agglutination

Indirect (passive) agglutination test that employs particles that are artificially coated with standard antigens or standard antibodies. The test is much more sensitive than direct one and allows detection of fewer antibodies or antigens (or small antigens).

These carriers include:

1. Red blood cells (Indirect (passive) gemagglutination test);
2. Polystyrene latex (Indirect (passive) latex agglutination test);
3. Bentonite;
4. Charcoal.

Accordingly to position – what is standard (antigen or antibody) –the reaction can be used for revealing unknown antibody and antigen.

Positive result of the indirect (passive) gemagglutination test looks like **umbrella** in the bottom of polysterol well at the microtiter plate.

Negative result shows red dot (button).

The employed particles have big sizes, much bigger than bacterial cells have, that is why these reactions are much more sensible, easy visible, than agglutination of bacterial cells. Standard substances for these reactions (artificially connected on the particles) are soluble, with small size.

3.4. REACTIONS BASED ON THE PHENOMENON OF PRECIPITATION: RING PRESCRIPTION, FLOCCULATION, GEL PRECIPITATION. PRACTICAL USE

When **a soluble antigen** combines with its antibody of immune serum in presence of electrolytes (NaCl) at a suitable temperature and pH, the antigen antibody complex forms insoluble precipitate. The test is called precipitation phenomenon.

The size of the precipitinogen is much smaller than the particles for agglutination test, that is why precipitated complexes is not dropping in the bottom. In a liquid medium, the formation of a precipitation, hanging in liquid, will occur.

Ring precipitation test

The antigen solution is carefully layered over serum in a narrow tube without mixing the liquids. The reaction is visible as a white zone at the junction of two clear fluids.

The ring precipitation test is used for the identification of antigenic component of bacteria Bacillus anthracis (cause of the anthrax) in infected animal tissue (Ascoli ring precipitation test) and for serological detection of blood, serum, etc (for example, to reveal the food falsification).

Flocculation

Flocculation is variety of precipitation reactions in the liquid in which the antigen-antibody complexes (often exotoxin or toxoid is antigen here, and it interacts with antitoxic antibodies) form a visible precipitate (flocculats) throughout the liquid volume.

Both antigen and antibody can be subsequently diluted (serial dilution) for this reaction like for the tube agglutination reaction. Then the reaction can be characterized quantitatively – can be determined titter of the reaction.

Slide flocculation test (micro precipitation test)

When a drop of antigen and antiserum is placed on a slide and mixed by shaking, floccules appear.

The slide flocculation test is used for identification of bacteria, e.g. detection of group specific polysaccharides substance in streptococci in Lancefield grouping, etc.

Tube flocculation test

When the antigen and antiserum are placed into tube and mixed by shaking, floccules appear. Antitoxic serum of known activity (200 IU/ ml) is poured on the tubes in such quantities: 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml. Then, we add 2 ml toxoid in each tube and we placed all tubes in a water bath at 45^{o}C. After this procedure, we observe the appearance of opalescence in the initial tube. If we know the number of antitoxic serum units in the initial tube, we can account number of international unit (IU) of toxoid in the tube. We make the calculation of toxoid activity and conclusion. This way is used for titration of the toxoid (type of preventive vaccine) antitoxic serum (for treatment some of infectious diseases).

The tube flocculation test is used for the Kahn test for syphilis (demonstration of antibody in serum) and for standardization of toxins and antitoxins.

Gel precipitation

The main advantages of this method are:

3. The precipitate is relatively fixed by agar medium and is easily visible. If antigen or antiserum contains more than one factor, then each factor produces separate precipitin line. Antigens and antibodies can be compared for common antigenic determinants.
4. Diffusion of a substance is process depending of shape, size and weight of the substance. In the place of meeting of the antigen and antibody the interaction has place and precipitin formation.

Practical use of gel precipitation reaction is to reveal toxigenicity of Corynebacterium diphtheria (Elek's gel precipitation test).

The strip of paper is saturated with antitoxic serum.

The cultures 1-4 inoculated in form of the lines. The culture 1 is standard non-toxigenic, no exotoxin producing, without lines of precipitation. The culture 3 is standard toxigenic, exotoxin producing, with lines of precipitation. The cultures 2 and 4 are the test cultures.

Accordingly, to the presence or absence of precipitation is possible to conclude about toxigenicity of investigated culture.

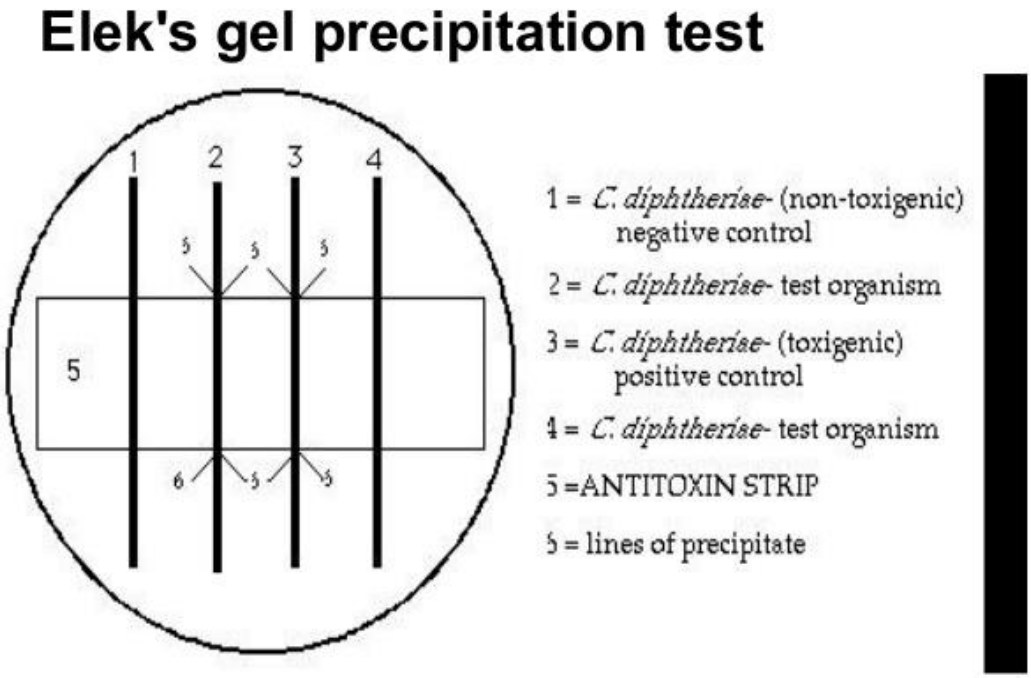


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Figure 8. Elek's gel precipitation test

3.5. NEUTRALIZATION TEST (TOXINS AND VIRUSES). PRACTICAL USE

An antitoxin is an antibody produced in response to a bacterial toxin or a virus that neutralizes the bacterial toxin and/or virus and therefore no harmful effects are produced.

Toxin neutralization

Bacterial exotoxins are good antigens. They induce the formation of antibodies, i.e. antitoxin. These antibodies protect from diseases like diphtheria and tetanus.

Anti-streptolysin O-test (ASO) in which antitoxin present in patient sera neutralize the hemolytic activity of the pathogen.

Toxin-antitoxin neutralization can be measured in vivo and in vitro. Flocculation reaction is an example of the in vitro test.

Virus neutralization

Viruses when mixed with immune serum lose their capacity to infect fresh host, e.g. vaccinia, influenza and poliomyelitis. Neutralization may be quantified on infected cell cultures or chicken embryos by different cytopathic effects.

3.6. REACTION OF IMMUNE LYSIS (BACTERIOLYSIS, SPIROHAETHOLYSIS, HEMOLYSIS)

Immune lysis reactions are antigens (cells) destruction under the action of specific antibodies (lysins) in the presence of the complement system proteins. It can be bacteriolysis, spirochaetolysis, hemolysis, etc. depend on the nature of target antigens (cells) in the lysis reaction. Fresh human immune serum can do lysis, because contains antibodies and complement. If serum was heated or stored some time, lysis can be only with adding of complement. Bacteriolysis and spirochaetolysis is not widely used in vitro. The hemolysis reaction is important as indicator reaction.

The hemolysins (specific protective antibodies) are artificially formed in the animal's blood serum after animal's immunization by erythrocytes. The haemolysins can destroy hemoglobin connection with erythrocytes' stroma in the presence of complement, and provide hemolysis reaction. The immune serum that contains haemolysins has the name of haemolytic serum. Such serums are produced by special laboratories. The power of haemolytic serum measured in titters – this is the maximum hemolytic serum dilution in volume 0.5 ml, that lead to full hemolysis of 0,5 ml 3% sheep erythrocyte suspension in the presence of 0.5 ml complement (in 1:10 dilution), and tubes incubation for 1 hour at 37^{o}C.

Hemolysis reaction is used as indicator (hemolytic system) for the complement-fixation test. This reaction name is immune hemolysis reaction. To carry out of the immune hemolysis reaction we need: 1) antigens – 3% suspension of erythrocytes; 2) antibodies – hemolysis serum against sheep erythrocytes; 3) complement – the serum of guinea-pig in 1:10 dilution; 4) isotonic sodium chloride solution (table 12).

Table 12. Scheme of the immune hemolysis reaction

№ tubes / Ingredient (ml)	test	controls		
	1	2	3	4
hemolytic serum	0.5	-	0.5	-
3% suspension of sheep erythrocytes	0.5	0.5	0.5	0.5
Complement (1:10)	0.5	0.5	-	-
Isotonic sodium chloride solution	-	0.5	0.5	1.0

The results of the reaction are read after test tubes incubation at 37°C for 45 min. Hemolysis should be present in the test tube (a result of specific interaction between hemolytic serum and erythrocytes in the presence of complement). Hemolysis should be absent in the control tubes: in the first tube (hemolytic serum control) complement is absent, in the second tube (complement control) hemolytic serum is absent, in the third tube (erythrocytes control) complement and hemolytic serum are absent.

3.7. COMPLEMENT FIXATION TEST. PRACTICAL USE

The complement fixation tests are best performed in reference laboratories where facilities exist for the careful standardization and control of reagents, which these tests require. The complement fixation tests are the technique that is used when serological reaction is not visible in agglutination or precipitation test, but can be demonstrated by use of CFT.

Complement-Fixation Test (CFT) is a very sensitive test and is capable of detecting 0.04 mg of antibody nitrogen and 0.1 mg of antigen. Principle of the test is the ability of antigen antibody complex to fix complement by classical pathway

Technique: Heat the patient's serums at 56°C for 30 minutes to destroy its own complement. Patient’s serum, complement (guinea pig serum) and standard bacterial or viral antigen are incubated at 37°C for one hour.

After that, sensitized sheep RBC are added as indicator system. The whole mixture is incubated at 37°C for 1 hour. If complement has been used up in first system, there would not be hemolysis, deposit of the red blood cells in the bottom instead. It means antigen-antibody reaction has taken place. Test is reported as positive. If sensitized RBC are lysed it means complement has not been fixed by first system and test is reported as negative.

CFT is used for serological diagnosis of such bacterial diseases, e.g, gonorrhoea, brucellosis; spirochaetal disease, e.g. syphilis (Wasserman reaction); rickettsia diseases, e.g. typhus fever; viral diseases like lymphogranuloma; parasitic diseases, e.g. kala azar, amoebiasis, toxoplasmosis.

3.8. REACTIONS USING LABELED ANTIGENS AND ANTIBODIES

Among microbiological methods immunological tests for diagnosis of infectious diseases the tests with labelled antigens or antibodies (immune fluorescence (IF) test, enzyme linked immunoassay (ELISA) and radioimmune analysis (RIA) are widely used. This is because of high sensitivity and specificity of these reactions, and because of the possibility to use it for express-diagnostics (very fast) of infectious diseases.

Serological reactions with labels based on the detection of interaction of antigen or antibody with immune complex (antigen-antibody) formation, where one of the reaction participants (standard) has labels. Labels can be detected visually or by special highly sensitive equipment. Serological reactions with labels allow to detect labelled substrate quantitatively in connection with unknown antigen or antibody, accordingly. As the labels are used: fluorescent dye in ultraviolet light (isothiocyanate fluorescein) in the immunofluorescence (IF) test; enzyme (peroxidase, alkaline phosphatase) that is detected by a colour change of the corresponding substrate in enzyme immunoassay (ELISA) test; isotope, that is detected by radiometry in radioimmune assay (RIA).

3.9. FLUORESCENT ANTIBODY TEST (FAT): DIRECT AND INDIRECT

The immunofluorescence (IF) test is based on the properties of fluorcscence antibody to connect specifically with homologous antigen and cause them to fluoresce in violet and ultraviolet parts of luminescent microscope spectrum. IF test is specific and sensitive. It is used mainly to identify the antigen and can be performed in several ways.

Direct IF test is based on the use of immunofluorescence sera against each investigated antigen. This unknown antigen should be insoluble particle bacterial cell or viral inclusion, for example. It is real staining of these unknown particles with fluorochrome as result of specific interaction antigen with antibody.

Indirect IF test is based on the use of two different sera. Initially, on the first stage, we use the unlabelled antibodies against the studied antigens and antigen-antibody complex is formed. On the second stage of reaction we treat this antigen-

antibody complex by immunofluorescence serum (this serum contains antibodies against gamma-globulins the same animal species, that is used for obtained unlabeled specific antiserum).

Conjugated primary antibodies are usually more expensive than their unconjugated counterparts. Secondary antibodies are relatively inexpensive compared to primary antibodies. Further cost savings may be made by using the same conjugated secondary antibody to detect different primary antibodies.

Sensitivity. The signal obtained in direct methods may seem weak when compared to indirect methods, as signal amplification provided by the use of secondary antibodies does not occur. Several secondary antibodies will bind to the primary antibody resulting in an amplified signal.

3.10. ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA): DIRECT, INDIRECT, SOLID-PHASE, COMPETITIVE

Enzyme-linked immunosorbent assay (ELISA) is based on the enzyme using as a label of antibodies. Enzyme can decompose substrates with formation of coloured products. They are direct and indirect variants of ELISA. Plastic microtitration plates, balloons, foil, tubes of various synthetic materials are used as solid-phase widely. Plastic microtitration plates are the mostly typical in standard set. Antigens or antibodies that adsorbed on the surface of such materials can keep their immunological specificity and ability to react in serological tests even in the dried state for a long time. Investigated substance is dropped in well with standard one, is incubated and washed out to remove nonconnected substunses.

The presence and quantity of antigen-antibody complex is detected by bound with the enzyme. It can be detected and evaluated by the intensity of staining visually after its incubation with the appropriate substrate and indicator of the biochemical reaction. ELISA results is recorded by photometer (reader).

Direct ELISA

Figure 29 illustrates the setup of direct ELISA. An antigen is immobilized in the well of an ELISA plate. The antigen is then detected by standard antibody directly conjugated to an enzyme such as HRP (Horse radish Peroxidase).

Direct ELISA detection is much faster than other ELISA techniques as fewer steps are required. The assay is also less prone to error since fewer reagents and steps are needed, i.e. no potentially cross-reacting secondary antibody needed. Although there are some disadvantages to this method. As the antigen immobilization is not specific, higher background noise may be observed in comparison to indirect ELISA

(see below). This is primarily because all proteins in the sample, including the target protein, will bind to the plate. As no secondary antibody is used there is no signal amplification, which reduces assay sensitivity. Finally, the direct ELISA technique is typically used when the immune response to an antigen needs to be analyzed (for scientific purpose).

Indirect ELISA

Standard antigen is adsorbed to a well in an ELISA plate. Detection is a two-step process. First, an unlabelled primary investigated antibody binds to the specific antigen. Second, an enzyme conjugated secondary standard antibody (anti-antibody) that is directed against the host species of the primary antibody is applied.

The indirect ELISA method has high sensitivity since more than one labelled secondary antibody can bind the primary antibody; it is more economical than the direct ELISA as fewer labelled antibodies are needed. Indirect ELISA delivers greater flexibility since different primary antibodies can be used with a single labelled secondary antibody ("anti-human"). Indirect ELISA assays take longer to run than direct ELISA since an additional incubation step for the secondary antibody is required. The indirect ELISA is most suitable for determining total antibody concentration in samples.

Sandwich ELISA

Sandwich ELISA require the use of matched antibody pairs (capture and detection antibodies). Each antibody is therefore specific for a different and non-overlapping region or epitope of the antigen. It is important that matched antibody pairs are tested specifically in sandwich ELISA to ensure that they detect different epitopes, to achieve accurate results. The capture antibody, as its name implies, binds the antigen that can then be detected in a direct ELISA or in an indirect ELISA configuration.

The procedure for a sandwich ELISA firstly requires the well of an ELISA plate to be coated with a capture antibody. The sample is then added, followed by a detection antibody. The detection antibody can be enzyme conjugated, in which case this is referred to as a direct sandwich ELISA.

Indirect sandwich ELISA

If the detection antibody used is unlabeled, a secondary enzyme-conjugated detection antibody is required. This is known as an indirect sandwich ELISA.

The key advantage of a sandwich ELISA is its high sensitivity; it is 2-5 times more sensitive than direct or indirect ELISA. Sandwich ELISA also delivers high specificity as two antibodies are used to detect the antigen.

Competition/Inhibition ELISA

The competition/inhibition ELISA, also known as a blocking ELISA, is perhaps the most complex of all the ELISA techniques. The competitive/inhibition ELISA is predominantly used to measure the concentration of an antigen or antibody in a sample by detecting interference in an expected signal output. Essentially, sample antigen or antibody competes with a reference for binding to a limited amount of labelled antibody or antigen, respectively. The higher the sample antigen concentration, the weaker the output signal, indicating that the signal output inversely correlates with the amount of antigen in the sample.

3.11. RADIOMUNOASSAY (RIA): COMPETITIVE, REVERSE, INDIRECT

This method is similar in principle to the non-radioactive sandwich ELISA method.

Radioimmune assay (RIA) is based on the use of radioactive isotopes as a label of one of the serological reaction components. The method is the most sensitive and can detect small amounts of reagents. RIA is used in both direct and indirect ways. The special radiometric equipment is required for the RIA assay and recording its results.

Radioimmunoassay (RIA) is a very sensitive and extremely specific in vitro assay technique used to measure concentrations of antigens by use of antibodies. As such, it can be seen as the inverse of a radiobinding assay, which quantifies an antibody by use of corresponding antigens. The RIA technique requires specialized equipment. It requires special precautions and licensing, since radioactive substances are used. The RAST test (radioallergosorbent test) is an example of radioimmunoassay. It is used to detect the causative allergen for an allergy.

Classically, to perform a radioimmunoassay, a known quantity of an antigen is made radioactive, frequently by labelling it with gamma-radioactive isotopes of iodine, such as 125-I, attached to tyrosine. This radiolabel antigen is then mixed with a known amount of antibody for that antigen, and as a result, the two specifically bind to one another. Then, a sample of serum from a patient containing an unknown quantity of the same antigen is added. This causes the unlabelled (or "cold") antigen from the serum to compete with the radiolabeled antigen ("hot") for antibody binding sites. As the concentration of "cold" antigen is increased, more of it binds to the antibody, displacing the radiolabeled variant, and reducing the ratio of antibody-bound radiolabeled antigen to free radiolabeled antigen. The bound antigens are then separated from the unbound ones, and the radioactivity of the free (unbound) antigen remaining in the supernatant is measured using a gamma counter.

This method can be used for any biological molecule on principle and is not restricted to serum antigens, nor is it required to use the indirect method of measuring the free antigen instead of directly measuring the captured antigen.

3.12. IMMUNOELECTRON MICROSCOPY

Immunoelectron microscopy emerges as a technique that links the information gap between biochemistry, molecular biology, and ultrastructural studies, by placing macromolecular functions within a cellular context. Immunoelectron microscopy is one of the best methods for detecting and localizing proteins in cells and tissues. This procedure can be used on almost every unicellular and multicellular organism, and often provides unexpected insights into the structure-function associations. The use of primary antibodies conjugated with gold particles allows high resolution detection and localization of a multiplicity of antigens, both on and within the cells.

The successful application of immunoelectron microscopy depends on the preservation of the protein antigenicity, the capacity of antibodies to infiltrate throughout the cell, and the specificity of recognition between antigen-primary antibodies.

Colloidal gold has become a very extensively used marker in microscopy. This tool has been applied to detect a vast range of cellular and extracellular constituents by using in situ hybridization, immunogold, lectin-gold, and enzyme-gold labelling. In addition to its use at light microscopy level, colloidal gold remains the label of choice in (transmission electron microscopy TEM) for studying thin sections, freeze-etch, and surface replicas, as well as in scanning electron microscopy. While conventional electron microscopy provides no information about specific molecules, immunogold labelling can help to connect a visible structure with a specific in situ localization and distribution of molecules at a high resolution. In this way, the use of the colloidal gold particles undoubtedly represents a significant event in the improvement of the immunochemistry method.

The use of immunoelectron microscopy can offer additional insights into the structure–function relationships.

3.13. MOLECULAR DIAGNOSIS. GENETIC METHODS: PCR, ELECTROPHORETIC ANALYSIS, IMMUNOBLOTTING, DNA-PROBE METHOD, DNA SEQUENCING, MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT (MALDI-TOF) MASS SPECTROMETRY, SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

The most complete way for molecular diagnosis with investigation of nucleic acids is present in the table:

Table 13. The main stages of molecular diagnosis techniques with investigation of nucleic acids

Denaturation, or separation, of the two strands of the DNA molecule
Polimerase chain reaction (PCR) for selective amplification of small quantity of genetic material with primers
Restriction
Agaros or poliacrilamid gel electrophoresis
Blotting on nitrocecculose or nylon filter
Adding of a probe marked with radioactive isotop, or fluorochrom, or enzime
Expose to x-ray film or immunofluorescent or ELISA and determination the result

All these stages are present in PCR, Reverse transcriptase polymerase chain reaction (RT-PCR) and Real-time PCR.

Terms

A primer is a short single-stranded nucleic acid utilized by all living organisms in the initiation of DNA synthesis. The enzymes responsible for DNA replication, DNA polymerases, are only capable of adding nucleotides to the 3’-end of an existing nucleic acid, requiring a primer be bound to the template before DNA polymerase can begin a complementary strand.

DNA probe is **single-stranded standard DNA** used to detect the presence of unknown **complementary single-stranded nucleic acid** sequences (target sequences) by hybridization.

DNA probes are usually labelled, with radioisotopes, epitopes, biotin or fluorophores for their detection.

Molecular hybridization is a basic property of nucleotide sequences. It is a phenomenon in which single-stranded deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) molecules anneal to complementary DNA or RNA.

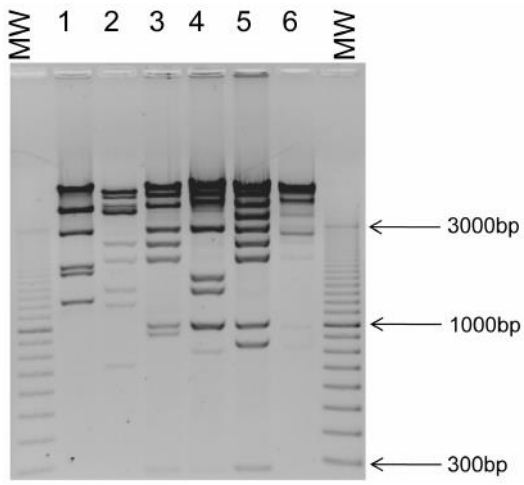
Restriction enzymes (endonucleases) are bacterial enzymes used by scientists to cut DNA molecules at known locations.

Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) is a type of polymorphism that results from variation in the DNA sequence recognized by one or more **restriction enzymes (endonucleases)**. These are bacterial enzymes used by scientists to cut DNA molecules at known locations. RFLPs (pronounced "rif lips") are used as markers on genetic maps. Typically, gel electrophoresis is used to visualize RFLPs. It can be distinguished by there electrophoretic mobility in poliacrilamid or agarose gel. RFLP can be used when there is sufficient amount of test material.

Table 14. The stages are present in Restriction Fragment Length Polymorphism test

Stages	Present or no
Denaturation, or separation, of the two strands of the DNA molecule	no
Polimerase chain reaction (PCR) for selective amplification of small quantity of genetic material with primers	no
RESTRICTION	**YES**
AGAROS OR POLIACRILAMID GEL ELECTROPHORESIS	**YES**
Blotting on nitrocecculose or nylon filter	no
Adding of a probe marced with radioactive isotop, or fluorochrom, or enzime	no
Expose to x-ray film or immunofluorescent or ELISA and determination the result	no



https://www.researchgate.net/figure/Restriction-analysis-of-phage-DNA-The-DNA-of-each-of-six-phages-from-pyophage-was_fig2_236604141

Figure 9. Restriction analysis of phage DNA. The DNA of each of six phages from pyophage was digested by Hind III and electrophorezed on a 0.8% agarose gel. From 1 to 6: P1-15 pyo , P8-13 pyo , P2-10 pyo , P3-

Fluorescent In situ hybridization (FISH)

Fluorescence in situ hybridization (FISH) is a cytogenic technique used for the detection and localization of RNA sequences within tissues or cells. It is particularly important for defining the spatial–temporal patterns of gene expression. FISH relies on fluorescent probes that bind to complementary sequences of the lncRNA (long non-coding RNAs (long ncRNAs, lncRNA), mRNA, or miRNA (microRNA) of interest. A series of hybridization steps are performed to achieve signal amplification of the target of interest. This amplification is then viewed using a fluorescent microscope. This technique can be used on formalin-fixed paraffin embedded (FFPE) tissue, frozen tissues, fresh tissues, cells and circulating tumor cells. These characteristics make FISH particularly good for studying lncRNAs, especially given their function is largely unknown.

Table 15. The stages are present in Fluorescent In situ hybridization (FISH)

Stages	Present or no
AN INTERPHASE OR METAPHASE CHROMOSOME PREPARATION IS USED.	**Yes**
SELECTIVE AMPLIFICATION OF SMALL QUANTITY OF GENETIC MATERIAL WITH PRIMERS	**Yes**
Restriction	no
Agaros or poliacrilamid gel electrophoresis	no
Blotting on nitrocecculose or nylon filter	no
EDDING OF RNA or DNA PROBE MARKED WITH	**Yes**

radioactive isotop, or **FLUOROCHROM,** or enzime	
EXPOSE TO X-RAY FILM OR IMMUNOFLUORESCENT OR ELISA AND DETERMINATION THE RESULT	**Yes**

Polymerase chain reaction (PCR)

Polymerase chain reaction is a method rapidly make millions to billions of copies of a specific DNA sample (make amplification), allowing to take a very small sample of DNA and amplify it to a large enough amount to study in detail.

Table 16. The stages are present in Polymerase chain reaction (PCR)

Stages	Present or no
DENATURATION, OR SEPARATION, OF THE TWO STRANDS OF THE DNA MOLECULE	**Yes**
POLIMERASE CHAIN REACTION (PCR) FOR SELECTIVE AMPLIFICATION OF SMALL QUANTITY OF GENETIC MATERIAL WITH PRIMERS	**Yes**
RESTRICTION	**Yes**
AGAROS OR POLIACRILAMID GEL ELECTROPHORESIS	**Yes**
BLOTTING ON NITROCECCULOSE OR NYLON FILTER	**Yes**
EDDING OF A PROBE MARCED WITH RADIOACTIVE ISOTOP, OR FLUOROCHROM, OR ENZIME	**Yes**
EXPOSE TO X-RAY FILM OR IMMUNOFLUORESCENT OR ELISA AND DETERMINATION THE RESULT	**Yes**

PCR is a three-step process that is carried out in repeated cycles.

1. The initial step is the **denaturation, or separation**, of the two strands of the DNA molecule. This is accomplished by heating the starting material to temperatures of about 95 °C. Each strand is a template on which a new strand is built;
2. In the second step the temperature is reduced to about 55 °C so that the primers can anneal to the template;
3. In the third step the temperature is raised to about 72 °C, and the DNA polymerase begins adding nucleotides onto the ends of the annealed primers.

At the end of the cycle, which lasts about five minutes, the temperature is raised and the process begins again. The number of copies doubles after each cycle. Usually from 25 to 30 cycles produce a sufficient amount of DNA.

Immunoblotting

Blotting Techniques are used in molecular biology for the identification of proteins and nucleic acids and is widely used for diagnostic purposes. This technique immobilizes the molecule of interest on a support, which is a nitrocellulosic membrane or nylon. It uses **hybridization techniques** for the identification of the specific nucleic acids and genes. The blotting technique is a tool used in the identification of biomolecules such as DNA, mRNA and protein during different stages of gene expression. Protein synthesis involves expression of a DNA segment which gets converted to mRNA to produce the respective protein. Molecules such as DNA, RNA and proteins are subjected to biochemistry analysis, which are separated using blotting techniques. In the case of a cell, these molecules are present altogether and hence with the help of blotting scientists are able to recognise a specific molecule out of all others. Blotting is performed by allowing a mixture of molecules of interest pass through a block of gel that separates the molecules based on their molecular sizes. The gel electrophoresis, which is generally applied for separation of DNA/RNA/protein and yields reproducible results attributed to their excellent resolving power.

Southern, Northern, Western, Eastern and South-western blots

Southern blotting was introduced by Edwin Southern in 1975 as a method to detect specific sequences of DNA in DNA samples. The other blotting techniques emerged from this method have been termed as **Northern (for RNA), Western (for proteins), Eastern (for post-translational protein modifications) and South-western (for DNA-protein interactions) blotting.**

Table 17. Types of blotting

Type of blotting	Substances
Western	**for proteins**
Northern	**for RNA**
Eastern	**for post-translational protein modifications**
Southern	**for DNA**
South-western	**for DNA-protein interactions**

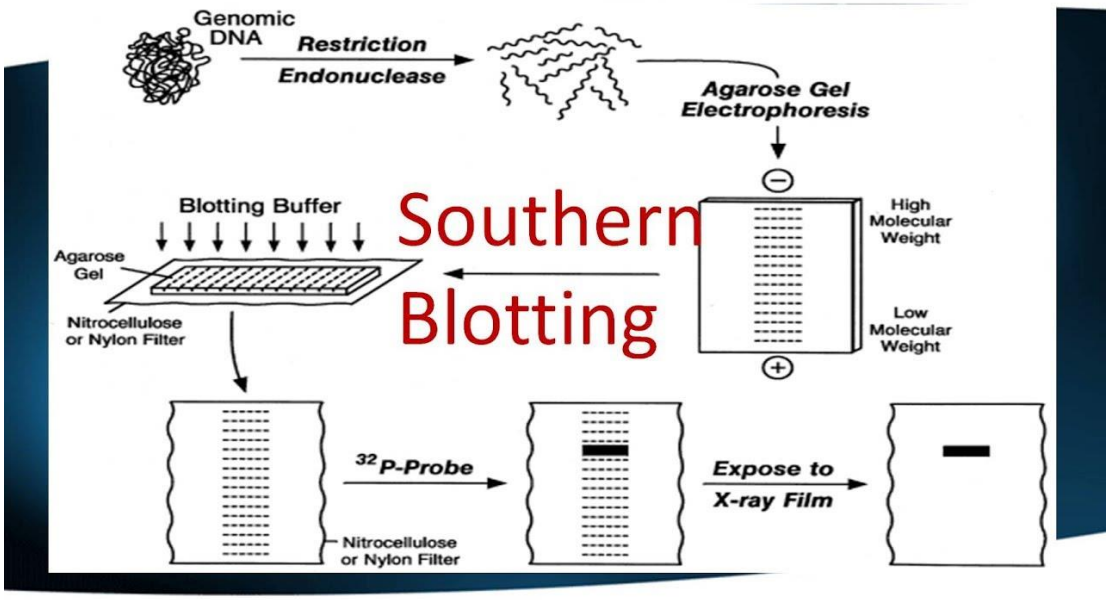
Immunoblotting is now widely used in conjunction with **two-dimensional polyacrylamide gel electrophoresis**, not only for traditional goals, such as the

immunoaffinity identification of proteins and analysis of immune responses but also as a **genome-proteome interface technique**.

Immunoblotting techniques use antibodies (or other specific ligands in related techniques) to identify target substances among a number of unrelated species. They involve identification of substances target via antigen-antibody (or protein-ligand) specific reactions.

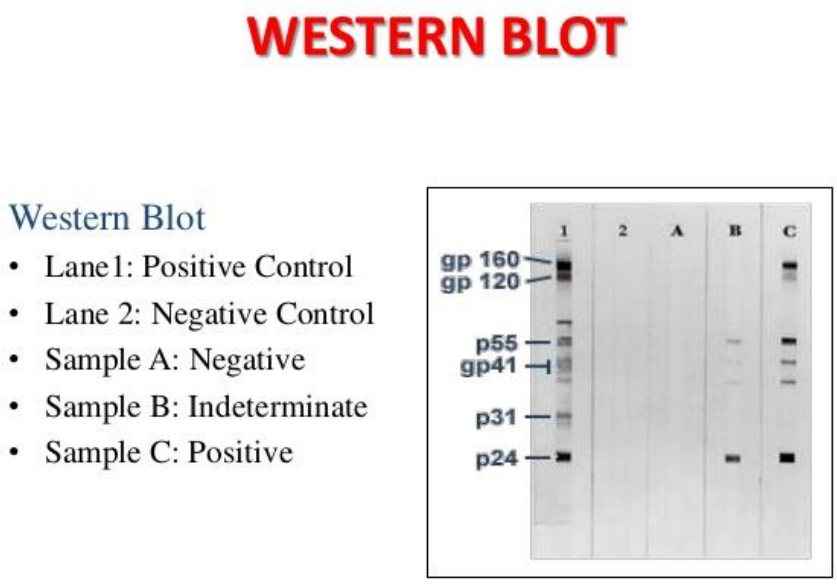
The hence processed molecules are required to be hard-pressed against a suitable membrane which will in turn transfer the molecules from the gel onto a suitable membrane (nylon, nitrocellulose or PVDF) via capillary action. After the molecules are transferred to the membrane their position does not change. Specific molecules can be detected amid the combination of molecules that are subjected to the separation.

In case of DNA and RNA the detection of specific sequences in the membrane are carried out via hybridization with nucleic acid labelled probes, which in the case of proteins is replaced by the use of labelled antibody probes. The initially developed protocols applied radioactive probes labelled with, radioactive isotopes for detection purposes via implementation of autoradiography procedures. In this process, using the pattern of decay emissions radiated from a radioactive material is applied to produce an image on an x-ray film that can also be made available as a digital image by application of scintillation based gas detectors or systems based on phosphorimaging. Keeping in mind the harmful effects of exposure to radioactivity other kinds of labelling systems have been developed which includes fluorescent and chemiluminescent reagents.



https://www.mybiosource.com/learn/southern-blotting/

Figure 11. Southern blotting



https://image.slidesharecdn.com/sayanti-140423040236-phpapp01/95/immunoblotting-techniques-8-638.jpg?cb=1398225926

Figure 12. Western blot

Reverse transcriptase polymerase chain reaction (RT-PCR) and Real-time PCR (RT-PCR)

RT-PCR is routinely used for viral RNA in research and clinical settings. Reverse transcription polymerase chain reaction (RT-PCR) is a laboratory technique combining reverse transcription of RNA into DNA (called complementary DNA or cDNA) and amplification of specific DNA targets using polymerase chain reaction (PCR). It is primarily used to measure the amount of a specific RNA. This is achieved by monitoring the amplification reaction using fluorescence, a technique called **real-time PCR or quantitative PCR (qPCR)**.

Dot blot

Dot blot is a simplified procedure in which protein samples are not separated by electrophoresis but are spotted directly onto membrane.

Dot blots are very similar to **Western blots** in that they involve the use of antibodies to identify a protein that has bound to a membrane. However, they do not require electrophoretic protein separation on a gel as the test sample is simply spotted on to the membrane and then detected.

Due to the lack of a protein separation step, dot blots cannot be used to determine the molecular weight of a protein or to discriminate between different protein forms (e.g. cleaved or phosphorylated proteins). Instead, dot blots are

convenient for estimating the protein concentration in crude preparations such as tissue culture supernatant or ascites, for determining whether an antibody based detection system will work effectively, or to identify an appropriate antibody concentration for Western blotting.

Table 18. The stages are present in Dot blot

Stages	Present or no
Denaturation, or separation, of the two strands of the DNA molecule	no
Polimerase chain reaction (PCR) for selective amplification of small quantity of genetic material with primers	no
Restriction	no
Agaros or poliacrilamid gel electrophoresis	no
BLOTTING ON NITROCECCULOSE OR NYLON FILTER	**YES**
EDDING OF A PROBE MARCED WITH RADIOACTIVE ISOTOP, OR FLUOROCHROM, OR ENZIME	**YES**
EXPOSE TO X-RAY FILM OR IMMUNOFLUORESCENT OR ELISA AND DETERMINATION THE RESULT	**YES**

The Main Steps of a Traditional Dot Blot Assay are as follows:

1. The protein is spotted onto a nitrocellulose or PVDF membrane, for example using a low-volume pipette or a pin tool
2. The membrane is blocked
3. Incubation with primary antibody
4. The membrane is washed to remove unbound antibody
5. Incubation with secondary antibody
6. The membrane is washed to remove unbound antibody
7. Detection – Usually colorimetric, fluorometric, chemiluminescent or using gold nanoparticles.

DNA sequencing

DNA sequencing is the process of determining the nucleic acid sequence – the order of nucleotides in DNA. It includes any method or technology that is used to determine the order of the four bases: adenine, guanine, cytosine, and thymine.

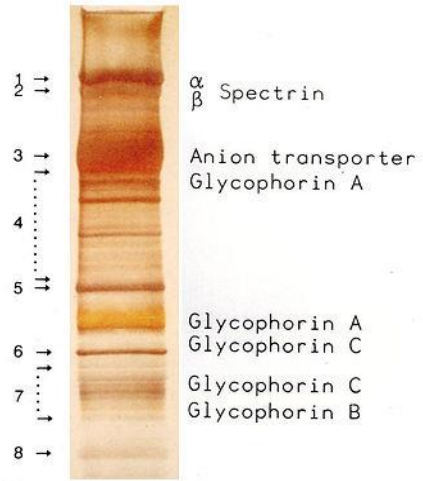
Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is a high throughput technology based on the comparison of the protein fingerprint obtained by microbial cells with a database of reference spectra by means of the use of various algorithms integrated in systems recently made commercially available.

In mass spectrometry, matrix-assisted laser desorption/ionization (MALDI) is an ionization method with use a laser energy-absorbing matrix to create ions from large molecules with minimal fragmentation. It has been applied to the analysis of biopolymers such as DNA, proteins, peptides and sugars and larger organic molecules like polymers, dendrimers and other macromolecules), which tend to be fragile and fragment when ionized by more conventional ionization methods.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE (sodium dodecyl sulphate–polyacrylamide gel electrophoresis), the use of sodium dodecyl sulphate (SDS, also known as sodium lauryl sulphate) and polyacrylamide gel largely eliminates the influence of the structure and charge, and proteins are separated solely based on polypeptide chain length. It uses sodium dodecyl sulphate (SDS) molecules to help identify and isolate protein molecules.



https://en.wikipedia.org/wiki/SDS-PAGE

Figure 13. Proteins of the erythrocyte membrane separated by SDS-PAGE according to their molecular masses

3.14. PRACTICAL USE OF FLUORESCENT ANTIBODY TEST (FAT), IMMUNO-ENZYME AND MOLECULAR DIAGNOSIS

The reactions are used in situations when classical methods like precipitation, agglutination, neutralizing and complement fixation tests are not effective. Because of big effectiveness the new reactions, it was named reactions for express diagnostics.

PART 4. VACCINES AND IMMUNE SERUMS

4.1. PRACTICAL USE OF ARTIFICIAL IMMUNITY. IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY

Pathogens of infectious diseases lead to the formation of an immune response of the patient's body to their **antigens**. The immune response promotes the elimination of the pathogen from the body of the patient. Man is recovering. Such an immune response is called **natural active**.

Every healthy adult's body has small amounts of thousands of different antibodies. Each one is highly specialized to recognize just one kind of foreign substance.

It is example of **adaptive (adaptive immunity)**. It can be **active and passive**.

Ready **immunoglobulins (antibodies)** a child gets from mother transplacental (Ig G) and with milk (the most abundant type is IgA) is called **natural passive**.

The role of antibodies is to bind with antigens and inactivate them so that other bodily processes can take over, destroy, and remove the foreign substances from the body. The knowledge has the fundamental meaning for practical use of the immunoglobulins.

Theoretical knowledge about mechanisms of the **natural immune protection** is used to reproduce **artificial immunity** to a pathogen. Artificial immunity can be created in **two ways**.

If the antigens of pathogen are introduced in a safe form, the immune system will form an immune response, just as it could have been done under conditions of the disease, artificial **active** immunity will be formed. Memory T- and B-cells if are present, create immune defence faster and stronger when real pathogen infects the host. It will be light form of disease or no disease.

Artificial immunity can also be **passive** when antibodies (immune serum or gamma globulins) are introduced into the body. Protection is provided without the participation of the patient's immune system. Passive artificial immunity will be formed.

The formation of artificial immunity can be used both for **prevention (prophylaxis)** and for **treatment (therapy)** of infectious diseases.

Immune prophylaxis is the elicitation of immune responses with the aim of inducing protection against the subsequent development of an infectious disease.

Immune therapy means the use of vaccines and sera as immunotherapeutic agents.

Practical use of active artificial immunity for prevention and treatment of infectious diseases

Active adaptive artificial immunity is created by vaccination – by artificial introduction in the host's body inactivated pathogen, or it's part, or it's pathogen inactivated exotoxin. The immunity is generated actively by the immune system of the host. It **prevents** of dangerous infectious diseases.

This way has **advantages and disadvantages**.

The advantage is in generation of strong immunity for long time, the disadvantage is that it cannot be working at the same time after injection.

The immunization has saved a billion lives and prevented countless illnesses and disabilities in the World. Vaccine-preventable diseases, such as measles, mumps, and whooping cough, are still a threat. They continue to infect children, resulting in hospitalizations and deaths every year. Because creation of vaccine-mediated protection is a complex challenge.

Effective plan vaccination can lead to creation of herd immunity, when even immunocompromised person, who cannot be vaccinated, can be protected from severe infection pathology by rupture of the epidemiological chain. It is a great advantage of preventive vaccination in it.

Vaccines application to stimulate the immune system as drugs **for chronic or latent infections treatment** is receiving considerable interest (herpes, leprosy, tuberculosis etc).

Practical use of passive artificial immunity for prevention and treatment of infectious diseases

Passive adaptive artificial immunity is created by injection of immune with ready antibodies in. In this case **the advantage is in generation of immediate defence, the disadvantage is that it cannot be immunity working for long time after injection**.

Immune serum may be used as a **preventive measure** after exposure to a pathogen to try to stop developing of illness (such as with measles, tetanus, hepatitis A, hepatitis B, rabies, or chickenpox).

Inoculation of **immune sera and immunoglobulins** for acute diseases **treatment** creates **immediate passive humoral immunity**, which can protect the body from infection or intoxication.

Antibody treatment may not be used for routine cases of these diseases, but it may be beneficial to high-risk individuals, such as people with immune system deficiencies.

4.2. MODERN CLASSIFICATION OF VACCINES

Drugs that are used to create artificial immunity are called immunobiological because they have a biological origin. They provide protection of the human body from infections precisely at the expense of the immune system that is reproduced.

Immunobiological drugs containing antigens and causing an active reaction of the immune system are called vaccines.

The list of modern **standard** vaccines includes **live, inactivated, chemical, anatoxins (toxoids), genetically engineered (recombinant), synthetic, anti idiotypical, DNA vaccines**.

Some vaccines are gathered together in so named **associated (combined) vaccines.**

It is possible to prepare an individual vaccine for a patient **(autovaccine).**

Adjuvants are nonspecific substances which can be added to some vaccines to increase their effectiveness.

Live (attenuated) vaccines

Live (attenuated) vaccines consist of viable strains of pathogenic microorganisms with the most reduced virulence, but with antigenic properties safe. Live vaccines create active immunity, similar to postinfectious. Most of live vaccines are attenuated – BCG, STI, EV, rickettsial, tularemial, brucellosis, but the first one was divergent.

Attenuation is sustained irreversible weakening of the virulence of pathogenic microorganisms, it is used for vaccine strains obtaining. Usually the mutant strains lack the genes coding for critical virulence factors, but remain highly immunogenic

For example, a new **bacterial typhoid vaccine** has been developed based at the use of an attenuated strain that grows poorly and is virtually nonpathogenic. Vaccination with live strain induced protective immunity in 90% of patients.

For **viral diseases prevention** Sabin's oral polio vaccine successfully developed on a scientific basis and the first to use the oral route for the immunization. This vaccine is a mixture of attenuated strains of the three known types of **poliovirus**. Other attenuated viral vaccines in current use include immune biological preparations against **mumps, rubella, measles, influenza, chicken pox and yellow fever vaccines.** Single divergent vaccine was successfully used for **small pox prevention**.

Killed (inactivated) vaccines

Killed (inactivated) vaccines consist of microorganisms that have expressed immunogenic properties, lost under the action of physical and chemical factors.

For **bacterial diseases prevention pertussis vaccine**, prepared with killed B. pertussis, the etiologic agent of whooping cough, is the prototypical killed vaccine. Killed vaccines, as usually, are reactogenic and have limited effectiveness, for example, **leptospirosis vaccine**.

Viral diseases prevention includes **tick-borne encephalitis** killed vaccine. The **influenza** vaccine is one of the most widely used inactivated viral vaccines. It contains one or two type A strains and one type B strain of influenza virus. The **killed polio vaccine** (Salk vaccine) is prepared by mixing the three known types of polio-vims after inactivation with formalin. Killed vaccines are the preference vaccines for immunocompromised children.

Some inactivated vaccines, autovaccines especially, are used for **chronic diseases therapy – gonorrhoea, staphylococcal infections.**

Subunit (chemical or component) vaccines

Chemical (component) vaccines contain the specific antigens that were extracted from bacteria and purified from ballast substances. Capsular polysaccharides of **S. pneumoniae, N. meningitides, Haemophilus influenzae type B (HiB)** and others are used as chemical vaccines.

Toxoids

Toxoids are inactivated bacterial exotoxins that have lost their active site but have maintained their immunogenic properties. Qualitatively they are medicine obtained from exotoxin by treatment with 0.3% solution of formalin 30 days at 37^{o}C. Administration of the toxoid induces the production of antibodies capable of neutralize the toxins by blocking their adsorption to cellular receptors. Therefore, **tetanus and diphtheria toxoids** are effective immunogens that induce long-lasting protection.

Genetically engineered (or recombinant) vaccines

It’s obtained on the basis of microbe genomes sequence detecting: genes that control the valuable antigenic determinants. They transfer gene into other microorganisms and clone them, promoting the expression of these genes by the new organism.

The ability to identify virulence mechanisms and manipulate with bacterial and viral genomes has resulted in the creation of a totally new class of vaccines, opening the door for multiple immunizations using a single construct.

Currently, it is prepared with **hepatitis B** antigens, produced by recombinant yeast cells transfected with plasmid constructs containing the HBsAg gene coding.

Large amount of HBsAg is obtained by disrupting the recombinant yeast cells and purified by chromatography.

Other way of recombinant vaccines production is using for relevant antigens of unrelated viruses or bacteria is added to the genome of a carrier (**vector**) organism. Live vector: recombinant vaccines virus (or adenovirus) has a large genome, allowing the preparation circumstance carrying multiple genes. A major problem with the use of a live vector vaccine is the fact that some live vaccines can be pathogenic in immunocompromised individuals.

Synthetic vaccines

A synthetic vaccine is a vaccine consisting mainly of synthetic peptides, carbohydrates, or antigens. They are usually considered to be safer than vaccines from bacterial cultures. Creating vaccines synthetically has the ability to increase the speed of production.

Anti-idiotypic vaccines

Anti-idiotypical vaccines have obtained from antibodies that are characterized by **similar structure** with the **antigen epitope** and active center of **antibodies anti-idiotype** (**anti-ids**). An antigen binding site in an antibody is a reflection of the three-dimensional structure of part of the antigen, that is of a particular epitope. This unique amino acid structure in the antibody is known as the **idiotype**, which can be thought of as a mirror of the epitope in the antigen. Antibodies can be raised against the idiotype by injecting the antibody into another animal. This gives us an anti-idiotype antibody and this, therefore, mimics part of the three-dimensional structure of the antigen, that is, the epitope. This can be used as a vaccine.

Table 19. Anti-idiotypic vaccines

Immunizing factor	**Resulting structure of paratope (idiotype of antibody)**
Real antigen (**epitope 1**)	Specific antibodies with specific **paratop 1** to antigen (**idiotype 1** of the antibodies)
Anreal antigen (**paratop 1** from antibody, **idiotype 1** of the antibody)	Specific antibodies with specific **paratop 2** to antigen (**idiotype 2** of the antibodies)
Spatial structure of the **epitope 1** is equal to the **paratop 2.** **Paratop 2** can be used for immunization against **epitope 1.**	

DNA vaccines

DNA vaccines are third generation vaccines. They contain DNA that codes for specific proteins (antigens) from a pathogen. The DNA is injected into the body and

taken up by cells, which normal metabolic processes synthesize proteins based on the genetic code in the plasmid that they have taken up. It has recently been reported that intramuscular injection of no replicating plasmid DNA encoding the hemagglutinin or nucleoprotein of the influenza virus or HBsAg of HBV elicits humoral or cellular protective reactions. It`s not understood how DNA becomes expressed and its message translated into viral proteins, but positive results obtained in this experiment have raised enormous interest in the scientific community.

Conjugated vaccines

A conjugate vaccine is a type of vaccine which combines a weak antigen with a strong antigen as a carrier so that the immune system has a stronger response to the weak antigen. Most commonly, the weak antigen is a polysaccharide that is attached to strong protein antigen. However, peptide/protein and protein/protein conjugates have also been developed.

Conjugated vaccines usually use polyribosylribitol phosphate (PRP) conjugated with protein carriers and conjugate vaccines for Haemophilus influenza and Neisseria meningitides (using outer membrane proteins, OMP) have already been developed.

Associated (combined) vaccine

Associated (combined) vaccine is a vaccine that is designed to protect against two or more diseases or against one disease caused by different strains or serotypes of the same organism. Therefore, combined vaccines contain two or more antigens that are either combined by the manufacturer or mixed immediately before administration. Such vaccines are in common use because they allow simultaneous administration of multiple antigens resulting in fewer injections and visits to the clinic. **“DT-based combined vaccines**”, which contain diphtheria toxoid (D) and tetanus toxoid (T) in combination with other antigens, have been in use since the middle of the 20th century.

Some of the earliest DT-based combined vaccines included an inactivated poliomyelitis vaccine (IPV) and/or a whole-cell **pertussis vaccine** (wP) component. Depending on the antigens included, these were called DT-IPV, DTwP-IPV, or DTwP. These were followed by combinations with various acellular pertussis antigens (aP) as an alternative to wP and with the addition of one or both of Haemophilus influenza type b conjugates (Hib) and hepatitis B surface antigen (HepB).

Adjuvants

Adjuvants are substances which enhance its antigenic efficiency by absorption delaying of an antigen or by other means. It is possible to boost the magnitude of an immune response by using of adjuvant to maintain the antigen in close proximity to immune cells and to keep the antigen from dissipating from the inoculation site. Different types of adjuvant are available. Such as (aluminium hydroxide gels, which keep the antigen from dissolving away) and microorganisms, e.g. whole B. pertussis. Two commonly used preparations are Freund's incomplete (antigen in an emulsion of mineral oil and water) and Freund's complete (complete because it adds mycobacterial antigens to the emulsion).

Auto vaccine

Auto vaccine is vaccine prepared by bacteria isolating from a sick individual, cultivating and further destroying of this strain by killing. Patient body's resistance to infection increases by this vaccine back administering to the patient. **Staphylococcal auto vaccine** is used usually at chronic infections.

4.3. METHODS OF MANUFACTURING, EVALUATING THE EFFECTIVENESS AND CONTROL OF VACCINES

Methods of manufacturing and evaluating the effectiveness of vaccines

Auto vaccine

Method of manufacturing, evaluating the effectiveness and control of the vaccines include stages of inactivated heating autovaccine preparing:

1. In order to check the purity, smears-preparations from culture isolated from the body of the patient with chronic staphylococcal infection should be prepared. In addition, paint using the Gram method. Accumulation of biomass of a microorganism culture.
2. Checking the purity of the culture (microscopy).
3. Preparation of any culture suspension in physiological solution. Prepare a washer from biomass by adding a physiological solution
4. Transfer 1 ml of the culture to a sterile test tube for further inactivation.
5. Inactivation of microbial culture. Warm the suspension of bacteria in a water bath for 1 hour at 80 °C.
6. Control of the sterility of the vaccine. Check the activated vaccine for sterility. Carry out suspension in a sterile nutrient medium. Seeds are placed in a thermostat, withstand 24 hours at 37^{o}C.

7. Titration (standardization) of the vaccine.
8. Preserving the vaccine.
9. Investigation of harmlessness.
10. Spilling the vaccine into an ampoule.

Control of vaccine

Control of vaccine by the basic principles are:

-To make control vaccine drugs on the sterility (the live vaccines should not contain other microorganisms).

-To make control vaccine drugs on the harmful absence. This control need to perform on the sensitive laboratory animals (according to the death or survival of the animals, clinical manifestations of infection or the presence of intoxication, bacteriological parameters and change the weight of animals).

-To make control vaccine drugs on the reactivity (on laboratory animals or, sometimes, on a limited number of people - volunteers). The assessment is carried out by temperature reaction of the organism, the development of inflammation at the injection site and other indicators.

-To check specific activity of vaccine preparations:

according to concentration of microbes;

according to antibody productions after the injection of animals;

according to the ability to prevent relevant to infection in animals after its infecting by virulent microorganisms

-To make control vaccine drugs on the oncogenicity. Corpuscular vaccine should be verificate into experimental animals.

4.4. VACCINE PROPHYLAXIS AND VACCINE THERAPY

Vaccine Prophylaxis can be considered under two directions:

- Routine immunization of the healthy infants and children that form part of basic health care – the creation of individual immunity and herd immunity.
- Immunization of individuals or selected groups exposed to risk of particular infection.

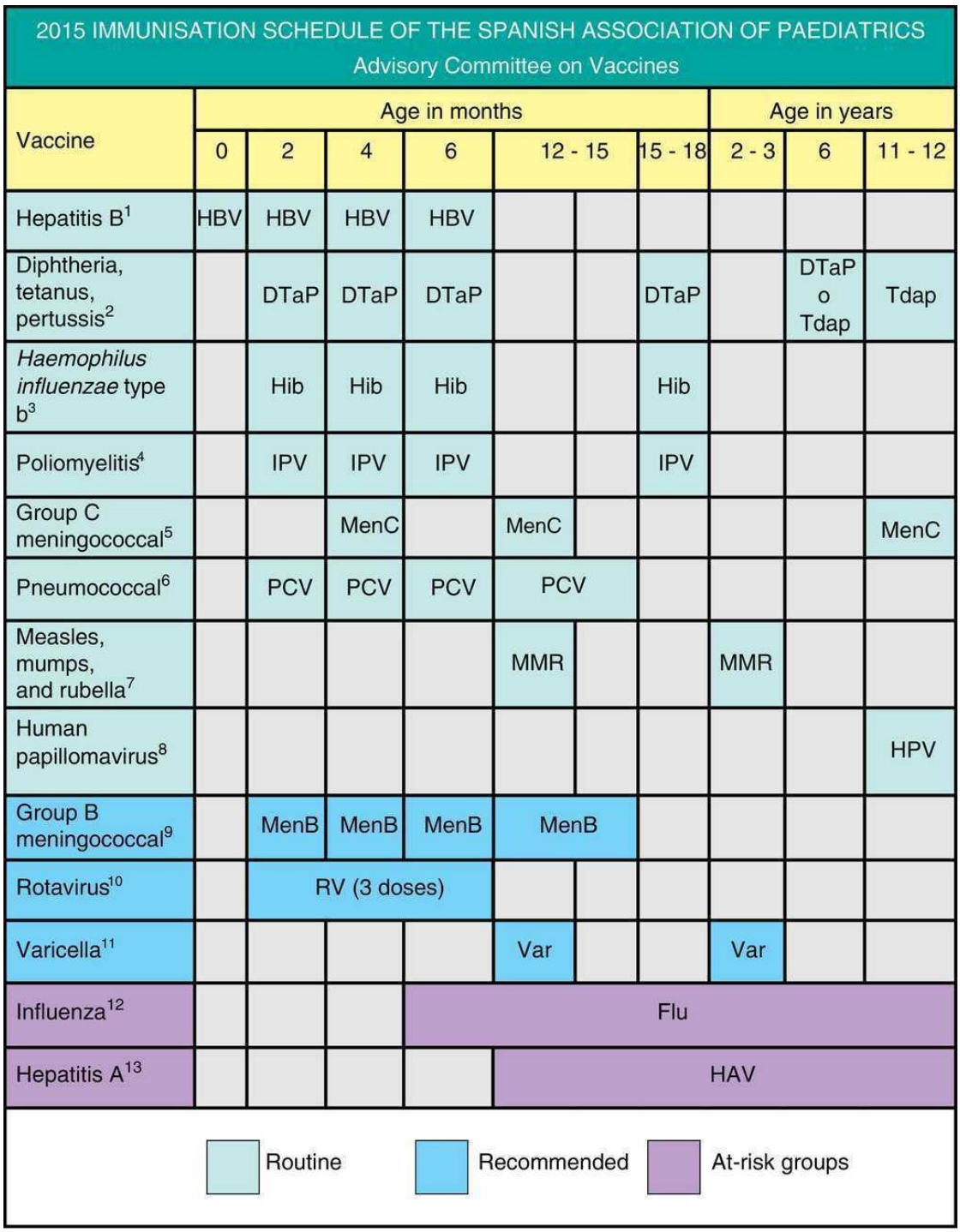


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Figure 14. Immunization Schedule (Calendar), example

In the USA and in India such vaccines abbreviachers are used:

DTP = diphtheria - tetanus - pertussis vaccine.

DTaP = diphtheria - tetanus - acelullar pertussis vaccine.

HepB = hepatitis B vaccine.

HiB = conjugated H.influenza vaccine.
MMR = measles - mumps - rubella vaccine.
OPV = oral polio vaccine.

Mechanism of vaccines action

After exposure to vaccine, the primary antibody response is formed and the cells of immunological memory (both B and T cells) appear.

When the same individual is exposed to the same antigen (vaccine) a second time, a somewhat different response, known as the secondary antibody response develops:

1. The lag or latent period is shorter;
2. There is a more rapid increase in the exponential phase;
3. The ultimate level of antibody is higher and persists longer than in the primary response;
4. There is more IgG than IgM in the antibody produced has occurred;
5. The amount of antigen needed to elicit the secondary response **(boster dose)** is smaller than that needed for the primary response;
6. The antibody produced has a higher mean affinity in the secondary response.

4.5. CONTRAINDICATIONS AND COMPLICATIONS AFTER VACCINE PROPHYLAXIS AND VACCINE THERAPY. PREVENTION OF COMPLICATIONS

Vaccines protect against diseases, but they can also cause a diverse range of mild to severe complications. While the most common complications, e.g., soreness, swelling, fever, and rash are mild, severe complications such as critical allergic reactions or seizures may also be scarcely induced. If these problems occur, they usually begin soon after the shot time and last 1 or 2 days. More serious problems following a flu shot can include small increased risk of Guillain-Barré Syndrome (GBS) - the acute immune-mediated polyneuropathies after inactivated flu vaccine.

4.6. SERUMS: CLASSIFICATION, PRINCIPLES OF THE OBTAINING, PURIFICATION AND CONTROL OF SERUMS AND IMMUNOGLOBULINS

Blood serum contains polyclonal antibodies and is used to pass of passive immunity to many diseases for treatment and prevention.

Table 20. Classification of serums

Sign	Examples
By origin	1. **Heterologous** are obtained by hyperimmunization of animals with vaccine drugs; 2. **Homologous** are serum of blood donors, volunteers, reconvalescents.
By appointment	1. **Therapeutic**; 2. **Prophylactic**.
By the mechanism of action	1. **Antitoxic** (anti-tetanus, anti-diphtherial); 2. **Antibacterial** (anti-plague, anti-anthrax); 3. **Antiviral** (anti-encephalitis, anti-pox).

Purification methods

Sera of blood contain many different organic substances with antigenic properties and it can result not only in defensive activity, but can lead to creation antiserum immune response. Additionally, many organic substances of sera possess of undesirable biological activity. It is why it is too important to purify the serum. The type and degree of purification depend upon the intended application for the antibody.

Purification methods range can be classified as follows:

1. **Physicochemical fractionation** - differential precipitation, size-exclusion or solid-phase binding of immunoglobulins based on size, charge or other shared chemical characteristics of antibodies in typical samples. This isolates a subset of sample proteins that includes the immunoglobulins.
2. **Class-specific affinity** - solid-phase binding of particular antibody classes (e.g., IgG) by immobilized biological ligands (proteins, lectins, etc.) that have specific affinity to immunoglobulins. This purifies all antibodies of the target class without regard to antigen specificity.
3. **Antigen-specific affinity** - affinity purification of only those antibodies in a sample that bind to a particular antigen molecule through their specific antigen-binding domains. This purifies all antibodies that bind the antigen without regard to antibody class or isotype.

4. **Size exclusion chromatography** is example of physicochemical method. **Dialysis, desalting and diafiltration** can be used to exchange antibodies into particular buffers and remove undesired low-molecular weight components. Dialysis membranes, size-exclusion resins, and diafiltration devices that feature high-molecular weight cut-offs can be used to separate immunoglobulins from small proteins and peptides.

Immunoglobulin

Immunoglobulins are biological drugs derived from immune serum, by purifying from the ballast protein fractions. The term "immunoglobulin" refers to the fraction of blood plasma that contains immunoglobulins, or antibodies. These immunoglobulins (Ig) in the serum or plasma are IgG, IgM, IgA, IgD and IgE.

Immunoglobulin therapy, also known as normal **human** immunoglobulin (**NHIG**), is the use of a **mixture of antibodies** (immunoglobulins) to treat a number of health conditions. These conditions include primary immunodeficiency, immune thrombocytopenic purpura, chronic inflammatory demyelinating polyneuropathy, Kawasaki disease, certain cases of HIV/AIDS and measles, Guillain-Barré syndrome, and in certain other infections when a more specific immunoglobulin is not available. The effects last a few weeks.

Hyperimmune globulin is prepared from the plasma of donors with high titters of antibody against a specific organism or antigen. Some agents against which hyperimmune globulins are available include hepatitis B, rabies, tetanus toxin, varicella-zoster, etc.

Monoclonal antibodies

Monoclonal antibodies (mAb or moAb) are antibodies that are made by identical immune cells that are all clones of a unique parent cell. Monoclonal antibodies can have monovalent affinity, in that they bind to the same epitope (the part of an antigen that is recognized by the antibody).

4.7. SEROPROPHYLAXIS AND SEROTHERAPY

Inoculation of immune sera and hyper immune immunoglobulins creates immediate passive humoral immunity, which can protect the body from infection or intoxication.

Immune sera against microorganisms and their toxins were widely used before advances in antibiotics and chemotherapy.

Antitoxic sera and, to a less extent, antiviral sera are still in use for prophylactics and therapy of diseases. The antitoxic sera in common use are

antidiphtheritic, anti-tetanus, and anti-gas gangrene sera. Passive immunization is the best method for diphtheria and tetanus preventing. The antidiphtheritic serum is rarely used for prophylactics now, but anti-tetanus serum (ATS) continues to be used extensively.

4.8. COMPLICATIONS IN SEROTHERAPY AND SEROPROPHYLAXIS. PREVENTION OF COMPLICATIONS

Passive immunization should be used only when absolutely necessary because of the risks involved such as developing **anaphylaxis, serum sickness, or a type III hypersensitivity reaction**. Anaphylaxis is an acute systemic (multi-system) allergic reaction in humans and other mammals.

Anaphylactic shock, the most severe type of anaphylaxis. Anaphylactic shock can lead to death in a matter of minutes if left untreated.

Symptoms of anaphylactic shock can include: skin reactions such as hives, flushed skin, or paleness, suddenly feeling too warm, feeling like you have a lump in your throat or difficulty swallowing, nausea, vomiting, or diarrhoea, abdominal pain, a weak and rapid pulse, runny nose and sneezing, swollen tongue or lips. **Blood pressure suddenly drops and airways narrowing, possibly blocking normal breathing. This condition is dangerous. If it is not treated immediately, it can result in serious complications and even be fatal.**

To prevent anaphylactic shock, an intracutaneous test is made previously by injecting 0.1 ml of 1:100 diluted sera into the flexor surface of the forearm If the reaction is negative (a papule no larger than 0.9 cm in diameter) the serum is injected (subcutaneously or intramuscular or even intravenous). If the intracutaneous test proves positive, it's necessary to make desensitization using the method proposed by Bezredko (fractional injection of the serum).

Prevention of serotherapy and seroprophylaxis complications is the method of administration of serum preparations by Bezredko:

1. Intracutaneously injected into the flexural surface of the forearm 0.1 ml of serum diluted 1: 100. The results are recorded in 20 minutes. The sample is considered negative if the diameter of the edema (hyperemia) at the injection site is less than 1 cm. The test is positive if the diameter is equal to 1 cm or more.

2. In the case of a negative skin test, an undiluted serum of 0.1 ml is administered subcutaneously in the area of the middle third of the shoulder. In the absence of a local or general reaction, after 30-60 minutes, intramuscularly administered the required dose of serum, heated to a temperature of 36^{o}C.

Serum sickness is a reaction to an antiserum derived **from an animal source**. Serum sickness typically develops up to ten days after exposure to the antiserum, and symptoms are similar to an allergic reaction.

Symptoms of serum sickness can include: **fever, general ill feeling, hives rash, itching, joint pain, rash, swollen lymph nodes.**

Part 5. IMMUNOLOGY OF ORAL CAVITY

5.1. MECHANICAL, CHEMICAL AND BIOLOGICAL FACTORS OF INNATE PROTECTION IN THE ORAL CAVITY (SALIVA AND ORAL FLUID, NORMAL MICROFLORA, LYSOZYME, OTHER ENZYMES OF SALIVA, COMPLEMENT, B-LYSINES, ETC.). FEATURES OF PHAGOCYTOSIS IN THE ORAL CAVITY

Innate immunity in the mouth

The major biological factors contributing to maintenance of oral health

Contributors to oral health

1. Integrity of oral mucosa
2. Lymphoid tissue
3. Saliva
4. Gingival crevicular fluid
5. Humoral and cellular immunity
6. Normal microflora (colonization resistance)

Oral mucosa

Oral health is dependent on the integrity of the oral mucosa, which normally functions as an effective barrier against microorganisms. If this barrier becomes compromised, for example in cancer patients with mucositis following chemotherapy, then infectious complications may ensue, including the risk of systemic infection. In addition, the oral mucosa is in continuity with a number of anatomical structures, such as the pharynx, which are vulnerable if the oral defences break down. A major area of risk is the junction between the gingiva and the tooth and the various forms of periodontal disease.

There are several factors, which may prevent penetration of intact oral mucosa by microorganisms. These include keratinisation in certain areas of the mouth, discharge of membrane-coating granules in the granular layer, formation of immune complexes by interaction of antigens with antibodies and the barrier function of the basement membrane. The small numbers of lymphoid cells adjacent to the basement membrane may help to deal with any organisms which pass through the overlying barriers.

Oral lymphoid tissues

Both extra-oral lymph nodes and intra-oral lymphoid aggregations are associated with the mouth.

Lymph capillaries originating superficially in the oral mucosa, gingivae and pulps of the teeth join to form larger lymphatics, which later join lymph vessels from a deep network in the other facial structures such as muscle of the tongue. These vessels drain into the submandibular, submental, upper deep cervical and retro-pharyngeal lymph nodes in an ordered fashion. Microbes that have passed through oral epithelium into the lamina propria may enter lymphatics directly or be transported to them by phagocytic cells. The antigen will thereby reach the anatomically neighbouring lymph nodes where an immune response may be elicited.

There are four types of lymphoid aggregations in the mouth. While the functions of the intra-oral lymphoid tissue are not fully understood, the tonsils are believed to guard the entry into the digestive and respiratory tracts, whilst the gingival lymphoid tissue responds to dental plaque. Secretory IgA, produced in the salivary glands, helps to prevent infection within the glands themselves but also protects the oral mucosa and tooth surfaces from microbial colonization.

Table 21. Summary of the collections of intraoral lymphoid tissue

INTRAORAL LYMPHOID TISSUE	
Palatine and lingual tonsils	Classical structure of lymphoid follicles B cells and perifollicular T cells Antigen penetrates through covering epithelium (no afferent lymphatics)
Salivary gland lymphoid tissue	Concerned mainly with synthesis of secretory IgA
Gingival lymphoid tissue	Plasma cells, lymphocytes, macrophages and polymorphs
Scattered submucosal lymphoid cells	Important in immunological response to dental plaque

Saliva

Saliva is a very important component of the oral defence, both by its mechanical washing activity and by means of the antimicrobial factors that it contains. Innate protection factors of saliva are: lysozyme; lactoferrin; peroxidase; beta-lysine; tetrapeptide sialin; acid glycoprotein; proteins rich in proline and histydine; nuclease; mucin; interferon. The important antimicrobial

Table 22. Summary of the major antimicrobial actions of saliva

ANTIMICROBIAL ACTIONS OF SALIVA	
Mechanical cleansing	Muscular movements, in conjunction with saliva, maintain hygiene in accessible areas of mouth Swallowed microbes are inactivated in the stomach
Lysozyme	Bactericidal, by splitting the bond between N-acetyl glucosamine and N-acetyl muramic acid in the cell wall. In saliva lysozyme enters the result of active secretion by mononuclear phagocytes and destruction of polymorphonuclear leukocytes, which contain it in large quantities.
Peroxidase	Heat-labile, anti-bacterial enzyme. As a result of the peroxidase system oxygen radicals with high reactive ability are generated and destroy lipid cell membranes of bacteria.
Beta-lysine	Produce action on the cytoplasmic membrane, causing autolysis of bacteria. This exhibit their bactericidal action against mainly anaerobic flora.
Lactoferrin	Heat-stable protein, bacteriostatic to many micro-organisms. Iron transport protein, bacteriostatic action is related to its ability to compete with bacteria for iron respiratory enzymes. Granulocytes are synthesized lactoferrins.
Acid glycoproteins	Agglutinins of nonimmunoglobulins nature that can block virus neuraminidase. They cause agglutination and loss of adhesive ability of viruses to the surface permissive cells.
Leucocytes	Saliva contains many leucocytes (99% polymorphs) Migrate from blood via gingival crevice
Sialin	This protein neutralizes the acidic products formed by microorganisms of the mouth, including those included in the composition of dental plaque. Thus, sialin has strong anticaries action.
Secretory IgA	IgA is the predominant immunoglobulin in saliva Produced by plasma cells within salivary glands Mainly in dimeric form, complexed with secretory component Functionally, secretory IgA prevents microbial adherence to host surfaces.

activities and components of saliva are summarized in Table 22. Secretory IgA is by far the most important immunoglobulin in saliva. IgA is secreted by salivary gland plasma cells, two molecules of which are combined by means of a J chain, which is

also secreted by local plasma cells. The resultant dimeric IgA is then complexed to the secretory component, synthesized by epithelial cells of the salivary acini, and the complete secretory IgA is transported into the duct lumen and then into the mouth. Secretory IgA is more resistant to proteolytic degradation than other immunoglobulins. It probably functions by combining with rnicroorganisms and preventing their adherence to host surfaces.

Gingival crevicular fluid

Blood components, including leucocytes, are able to reach the oral cavity via the flow of fluid through the functional epithelium of the gingival. The flow of this so-called gingival crevicular fluid (GCF) increases greatly with the inflammation accompanying periodontal disease. Complement components have been detected in GCF, suggesting that both the classical and alternative complement pathways may be activated in the gingival crevice. Other components include enzymes such as lysozyme, proteases and collagenases released by cells of both the host and bacteria. Specific proteases which inactivate IgA have been described.

The cellular component if GCF comprises mainly neutrophils, with small numbers of macrophages and B- and T-lymphocytes. These cells migrate continuously from the blood through the functional epithelium into the gingival crevice. Over 80% of neutrophils in the gingival crevice are functional and can phagocytose microorganisms.

It is clear, therefore, that tooth surface is influenced by both local salivary immune mechanisms, mediated largely through secretory IgA, and by systemic immunity involving all the varied immune components present in blood. The way in which these contributing factors interact to provide immunity within the oral cavity is illustrated in *fig. 8.*

The value of the oral cavity normal microflora

Normal flora is one of the factors of innate defence system. It has antagonistic properties against pathogenic and putrefactive microflora by producing acids, antibiotics, bacteriocines.

Participation in **colonization resistance**.

Can cause **autoinfection or endogenous infection**. When microbes-commensals appear in unusual places, they can cause the development of infection.

As a result of microbial decarboxylase and LPS released additional amount of histamine that can cause **allergic conditions**.

Is the repository and source of chromosomal and plasmid genes, including genes of drug **resistance to antibiotics.**

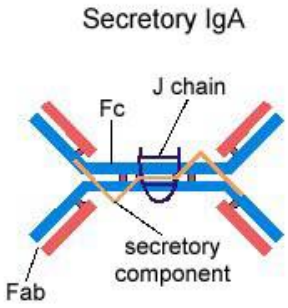
Features of phagocytosis in the oral cavity

Over 80% of neutrophils in the gingival crevice are functional and can phagocytose microorganisms. These cells migrate continuously from the blood through the functional epithelium into the gingival crevice. Saliva contains many leucocytes (99% polymorphs and small numbers of macrophages). Migrate from blood via gingival crevice. Special features of phagocytosis in the oral cavity may occur at autoimmune problems – periodontal diseases for example. In this case some side harmful effects of phagocytosis may be. Release of neutral proteases and reactive oxygen metabolites may lead to tissue damage.

5.2. IMMUNOGLOBULINS OF SALIVA. THE ROLE OF SECRETORY IMMUNOGLOBULINS

Immunoglobulins of saliva

Secretory IgA is the predominant immunoglobulin present in saliva. IgA is secreted by salivary gland plasma cells, two molecules of which are combined by means of a J chain, which is also secreted by local plasma cells (fig.15). The resultant dimeric IgA is then complexed to the secretory component, synthesized by epithelial cells of the salivary acini, and the complete secretory IgA is transported into the duct lumen and then into the mouth.



https://www.yandex.ua/yandsearch?clid=9582&text=Structure%20of%20secretory%20Ig%20A&l10n=ru&rdrnd=700496&lr=964&redircnt=1600937360.1

Figure 15. Structure of secretory Ig A

The role of secretory immunoglobulins

Secretory IgA is more resistant to proteolytic degradation than other immunoglobulins. It probably functions by combining with rnicroorganisms and preventing their adherence to host surfaces. IgA antibodies are important in resistance to infection of the mucosal surfaces of the body, particularly the respiratory, intestinal

and urogenital tracts. So it can be detected in gastrointestinal (GI) fluids, nasal secretions, saliva, tears and other mucous secretions of the body.
IgA acts as a protective coating for the mucous surfaces against microbial adherence or initial colonization. IgA can also neutralize toxin activity on mucosal surfaces.

Secretory IgA is also transferred via the milk, i.e., the colostrum, from a nursing mother to a newborn, which provides passive immunity to many pathogens, especially those that enter by way of the GI tract. The transfer of IgA via the milk lasts about six months in a woman and the infant encounters many infectious agents while thus partially protected. Under these circumstances the infectious agent might multiply, but only to a limited extent, stimulating the infant's own immune response without causing significant disease (e.g. poliovirus). The infant thus acquires active immunity while partially protected by maternal immunity.

5.3. USE OF SEROLOGICAL REACTIONS FOR THE DIAGNOSIS OF INFECTIOUS DISEASES DURING A SPECIFIC PROCESS IN THE ORAL CAVITY (SYPHILIS, GONORRHEA, DIPHTHERIA, HERPETIC INFECTION, ETC.)

Serological method is based on the detection of specific antibodies in the serum of patients with a particular pathogen. Therefore, we can use serological methods for diagnosis of infectious diseases under specific localization process in the oral cavity. In these cases, we need to take 10 ml of blood from patient elbow vein and prepare serum. For the tests based on complement binding principle serum must be warmed at 60 C for patient’s own complement destroying.

Wasserman test with specific and nonspecific (cardiolipid complex) known antigens is used for secondary syphilis diagnostics. Reuter`s complement binding test is used for chronic gonorrhoea diagnostics.

For herpetic infection diagnostics and other diseases caused by viruses, Mycoplasma and Chlamydia serum is drawn in the acute phase and compared with a sample drawn later in the convalescent phase. Antibody levels can be expressed as titter (i.e., the sample is tested "neat" or undiluted or at 1:10 and then diluted serially twofold, to 1:20, 1:40, 1:80, etc); the highest or last dilution giving a "positive test" is the one reported out as the titter level. (How "positive test" is defined depends on the specific assay). A change in titter of fourfold or greater between acute and convalescent samples is considered diagnostic of infection. Thus, if the overall antibody level is 1:20 in the acute phase and is 1:160 two weeks later in an assay for antibodies to *Mycoplasma pneumonia*, it can be said that the patient's pneumonia was due to that organism. For organisms that are difficult to culture, such serologic testing may be the only way to make a specific diagnosis.

Even better, if you can test for IgM and IgG antibodies, you can document the fact that the infection is recent.

2. What pattern of IgM and IgG antibodies might you expect in a recent infection? In an infection of long duration?

Another useful strategy is to test the fluid obtained from the site of inflammation. Suppose you think the patient has syphilis affecting the brain. Do a spinal tap (lumbar punction) and analyze the spinal fluid for antibodies to *Treponema pallidum*.

3. Is Lyme disease a possible explanation for your patient's arthritis?

Look at the joint fluid (synovial fluid) levels of specific antibodies and compare with the levels present in serum in order to demonstrate relative concentration of the specific antibodies at the site of inflammation. This then allows one to make a diagnosis. When the same individual is exposed to the same antigen a second time, is somewhat different response, known as the secondary immunoglobulin (or antibody) response develops. First, the lag or latent period is shorter. Second, there is a more rapid increase in the exponential phase. The ultimate level of antibody is higher and persists longer than in the primary response. Third, there is more IgG than IgM in the antibody produced; i.e. isotype switching, which is likely T-cell dependent, has occurred. (There is an exception to this rule: T-dependent antigens typically produce a smaller amplitude response in the primary response and little if any IgG, even in the secondary response). Fourth, the amount of antigen needed to elicit the secondary response is smaller than that needed for the primary response. Finally, in certain circumstances, the antibody produced has a higher mean affinity in the secondary response.

4. Why should the mean affinity increase in the secondary response?

There are probably two mechanisms behind this fact. First, let us consider an experiment comparing a large second inoculant with a small dose. The smaller dose leads to the development of serum antibodies with a higher affinity than does the large dose. The explanation for this is that memory cells are produced during the primary response. These cells persist for months to years after the primary exposure. They have on their surface different isotypes of antibody against the inoculant, each antibody with its own affinity for the antigens. Once the inoculant is given again and the antigen is bound to the surface immunoglobulin, these cells spring into action, making the antibody more rapidly than would naive cells. If a small second dose is given, only those cells with antibody of high affinity will bind the antigen and be stimulated to make immunoglobulin. Thus, a small dose essentially selects for the production of high affinity antibodies. If a high dose is given, cells with lower affinity surface immunoglobulin will also be activated. A large dose causes the production of a more heterogeneous population of antibody, but a population with a lower mean affinity. If the second inoculation follows the initial exposure after too

short a period of time, there will be a small antibody response to the second inoculation. The reason for this are two folds. First, if there is still serum antibody present from the initial response, the second dose of antigen will be bound by free antibody and fail to reach the surface immunoglobulin on memory cells. Second, memory cells may not yet have formed. If the second inoculation is given too long after the primary exposure, the memory cells, which have a finite life-span, may have died.

5.4. MECHANISMS FOR THE FORMATION OF SPECIFIC IMMUNITY OF THE ORAL CAVITY

The oral cavity of mucosal immune system mounts immune responses through immune cells residing in mucosal compartments (fig.16). T lymphocytes residing in the mucosa play important roles in mucosal immunity and tolerance. In addition, T cell deficiency or defects in T cell function are associated with several oral mucosal diseases. However, the phenotype and function of T cell subsets that reside in the oral mucosa remain largely undetermined. Thus, it is crucial to understand the diversity and functions of mucosal T cell subsets in healthy and pathological conditions.

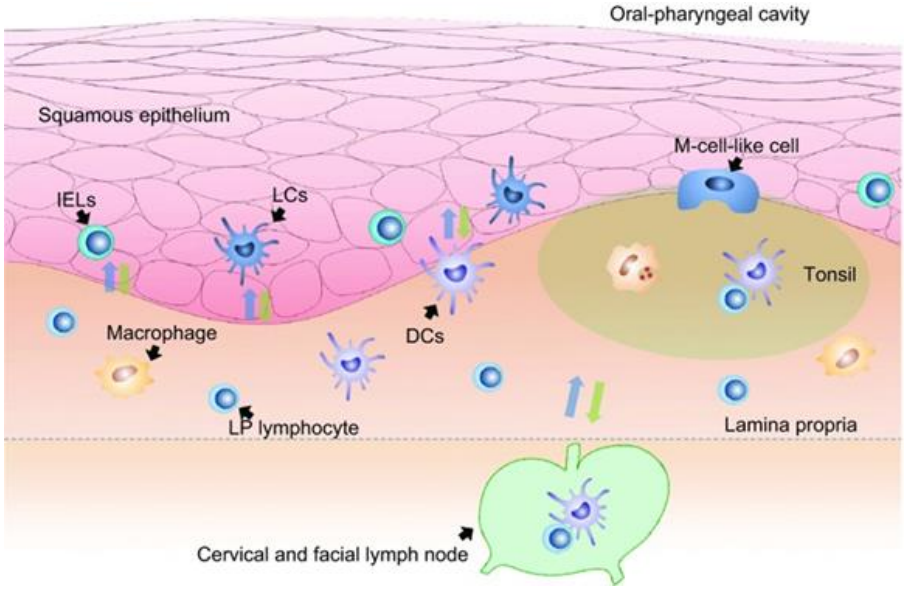


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Figure 16. The structures and immune cells in the oral immune system

Conventional T cells in the mucosa can be classified as either major histocompatibility class II (MHC II)-restricted and αβ T cell receptor (TCR)-expressing CD4 T cells (CD4 T cells, or TH cells) or MHC I-restricted and αβ TCR-

expressing CD8T cells. These T cells develop in the thymus and migrate into mucosal effecting sites after encountering antigen stimuli in lymphoid tissues.

Compartmentalized immune cells, such as lymphocytes, undertake the elimination of foreign antigens. After antigen uptake in the epithelium, they migrate lymph nodes and initiate the adaptive immune responses by inducing T cell proliferation and differentiation.

In the oral cavity, the mucosa is covered with saliva that contains immunoglobulins, such as secretory IgA, antimicrobial peptides such as defences, and enzymes secreted by salivary glands.

The cells are found to play an important role in the recruitment of neutrophils and osteoclasts. Consequently, the gingival barriers are destroyed together with the retraction of gingiva and destruction of alveolar bone. The chronic inflammatory disease, is characterized by massive lymphocyte infiltration in the LP and results in chronic destruction of the epithelium basal layer. Th1 and Th2 cells contribute to inflammation and mucosal lesion formation in oral lichen planus (OLP). Pro-inflammatory cytokines, including IL-6, IL-17 and TNF-α, are increased in the saliva and serum of OLP patients. On the contrary, TGF-β is decreased in the serum of OLP patients compared with that of healthy individuals.

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ILLUSTRATIONS IN COLOR

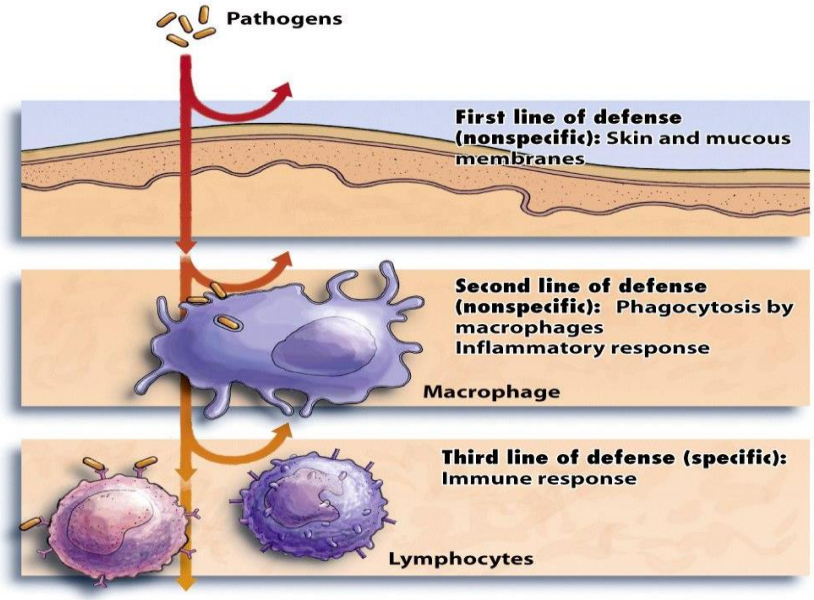

Figure 1. Lines of defence

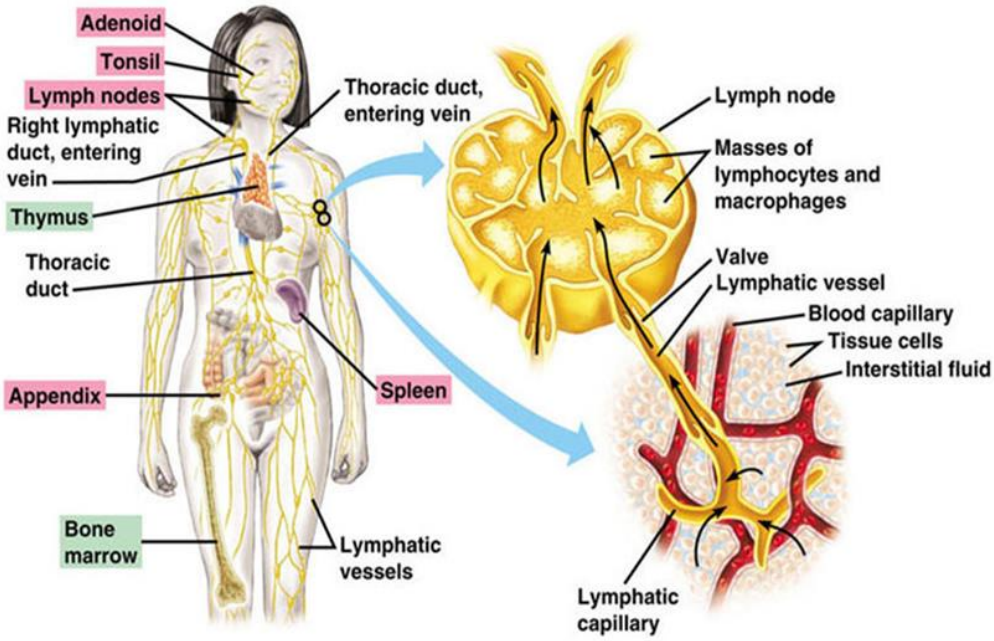

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Figure 2. Immune system

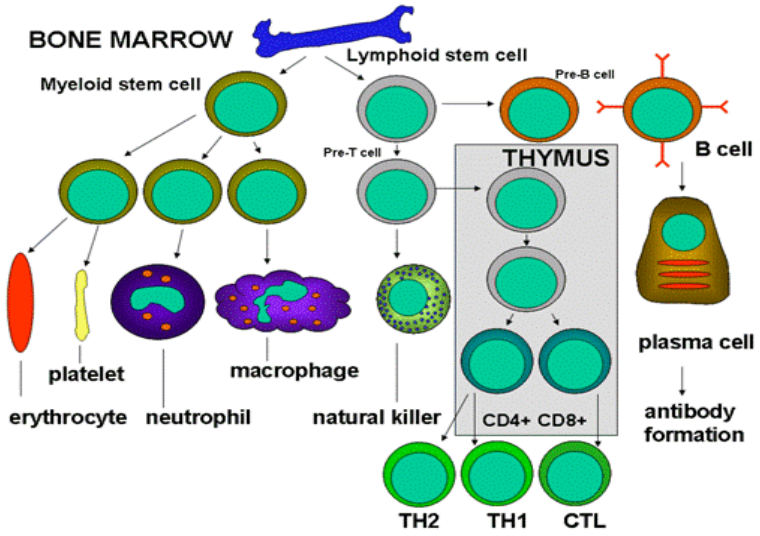

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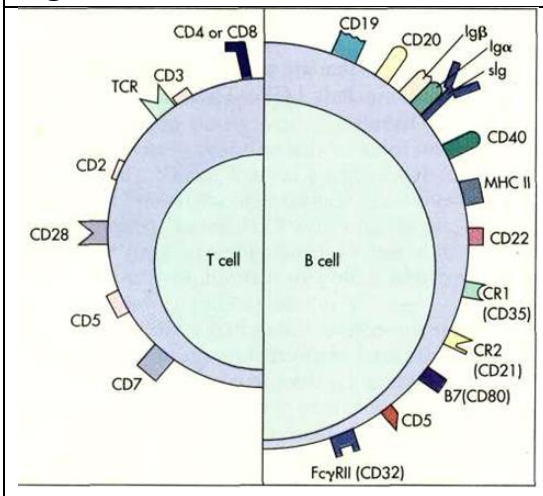
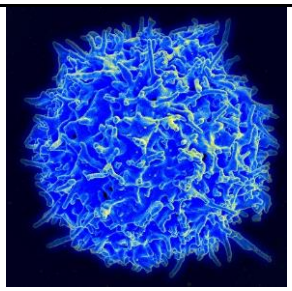
Figure 3. Division of the immune cells by their activity (innate and adaptive immunity)



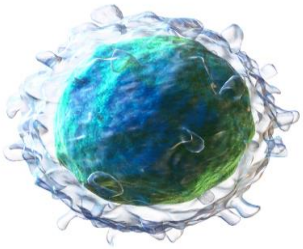
https://www.google.com/search?q=Surface+markers+of+human+B-+and+T-cells+images&tbm=isch&ved=2ahUKEwjglIm6zZzpAhVJyioKHZ74C9QQ2-cCegQIABAA&oq=Surface+markers+of+human+B-+and+T-cells+images&gs_lcp=CgNpbWcQDFCQ_gxYkP4MYK-QDWgAcAB4AIABkgGIAZIBkgEDMC4xmAEAoAEBqgELZ3dzLXdpei1pbWc&sclient=img&ei=FkyxXuCxHsmUqwGe8a-gDQ

Figure 4. Surface markers of human B- and T-cells



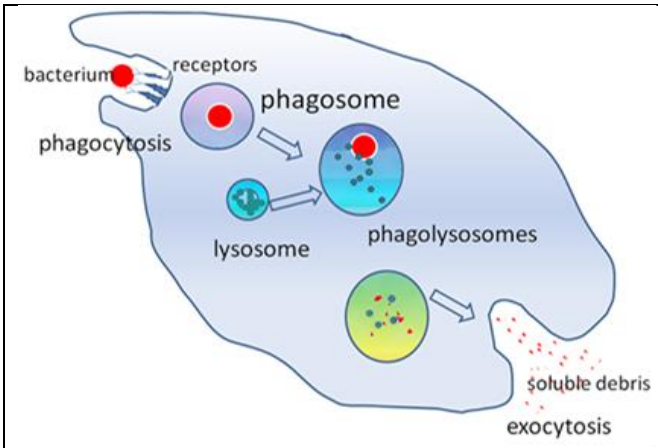
https://en.wikipedia.org/wiki/T_cell#/media/File:Healthy_Human_T_Cell.jpg

Figure 5. Scanning electron micrograph of a human T lymphocyte (also called a T cell) from the immune system of a healthy donor



https://upload.wikimedia.org/wikipedia/commons/7/7c/Blausen_0624_Lymphocyte_B_cell_%28crop%29.png

Figure 6. 3D rendering of a B cell



GrahamColm at English Wikipedia [CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0)

Figure 7. Completed phagocytosis

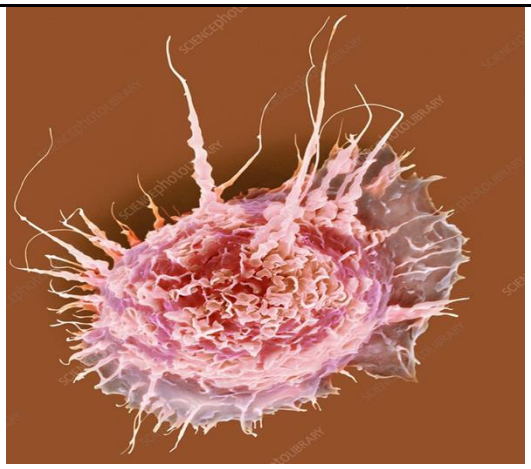


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Figure 8. Alveolar macrophage

https://www.sciencephoto.com/media/890939/view/macrophage-engulfing-tuberculosis-bacteria-illustration

Figure 9. Macrophage engulfing tuberculosis bacteria

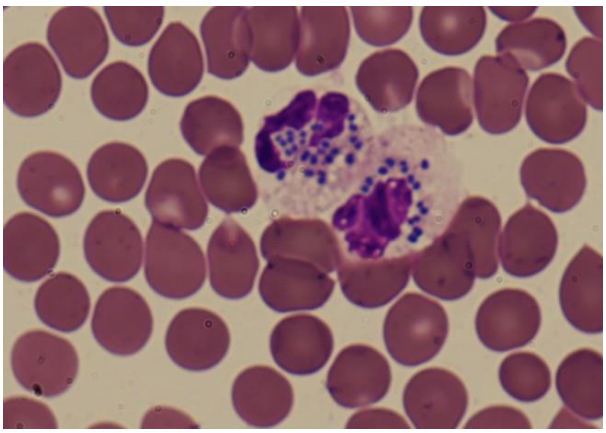

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Figure 10. Neutrophil phagocytosis in blood

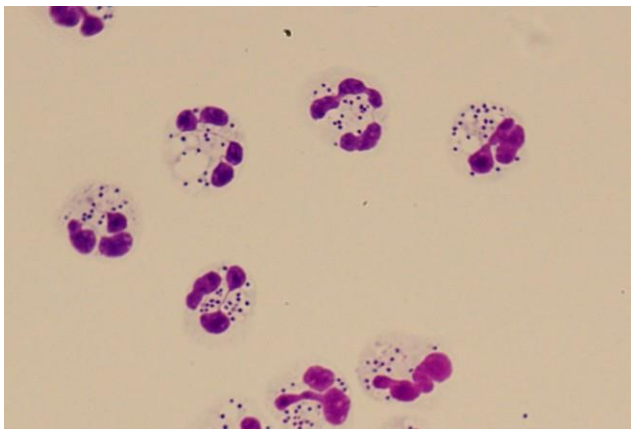

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Figure 11. Neutrophil phagocytosis in pus

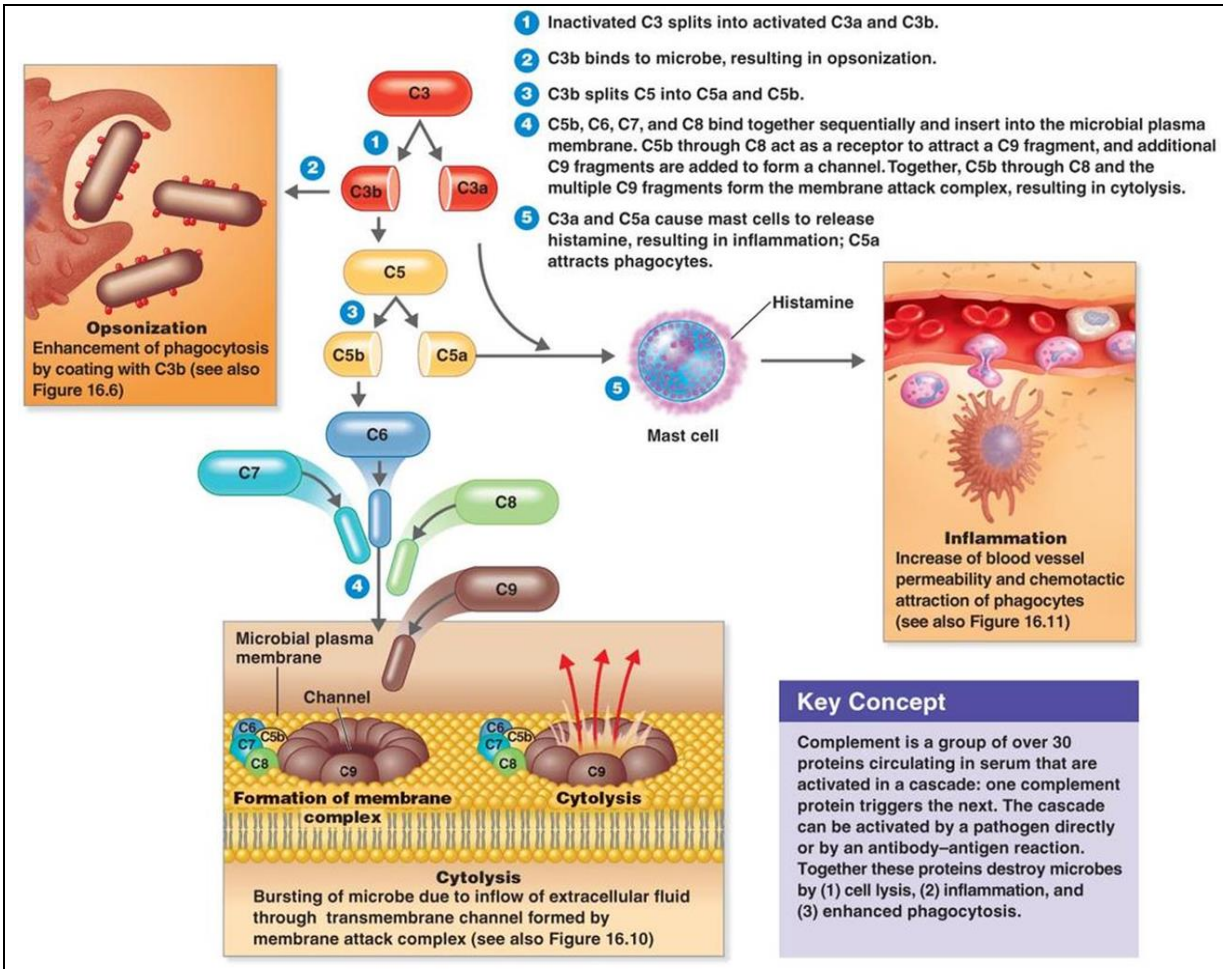

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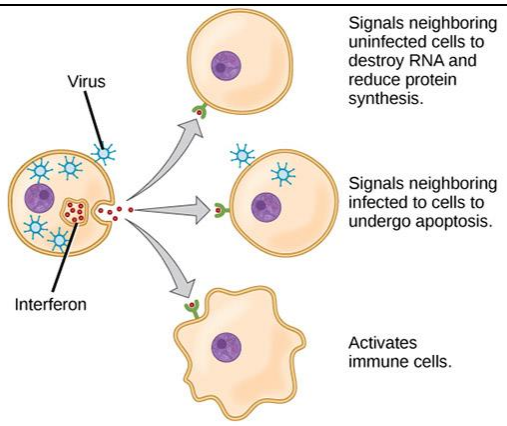
Figure 12. The role of complement in cell lysis, inflammation and enhanced phagocytosis

IFN Types	IFN Categories	Receptors	Prototypic cell of Origin
Type-I	Alpha (α)	I	Leukocyte
	Beta (β)	I	Fibroblast
	Omega (ω)	I	Leukocyte
	Tao (τ)	I	Ovine Trophoblast
Type-II	Gamma-γ	II	T-Cell NK-Cell

https://pt.slideshare.net/drchinmaya/interferon-29767706/5

Figure 13. Types of interferons



https://blogs.lse.ac.uk/latamcaribbean/2020/03/18/cuba-and-coronavirus-how-cuban-biotech-came-to-combat-covid-19/

Figure 14. Antiviral activity of interferon

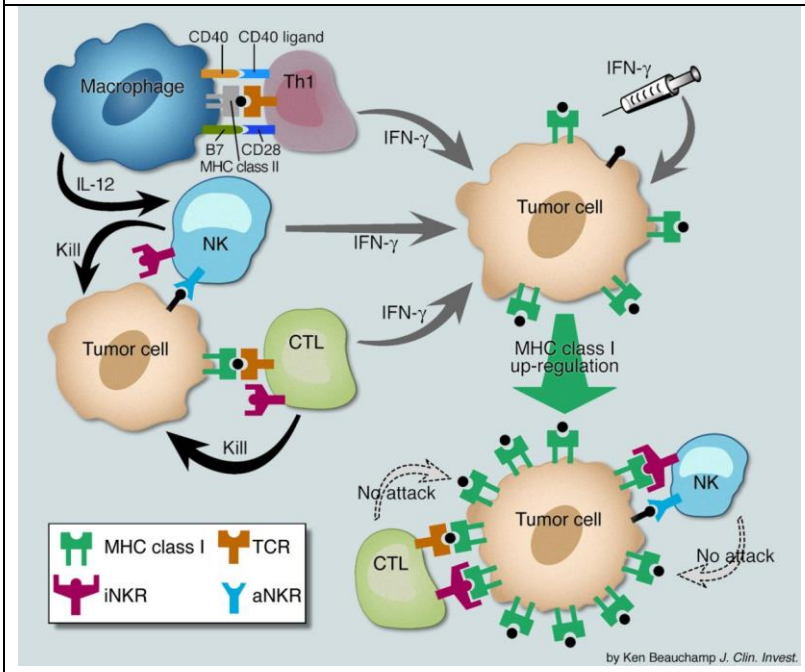


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Figure 15. Antitumor activity of interferon

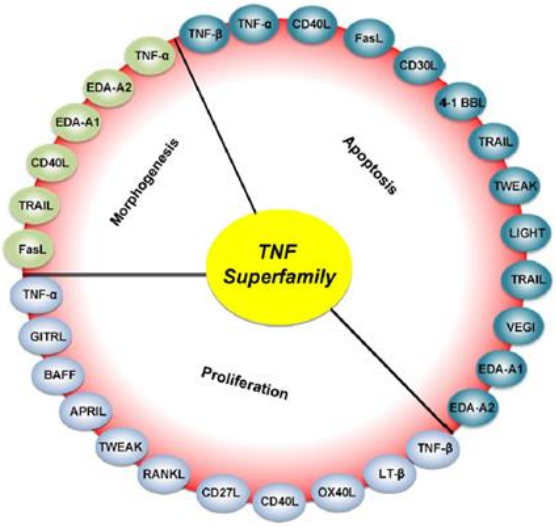

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Figure 16. Effects of TNF in interaction with another factors

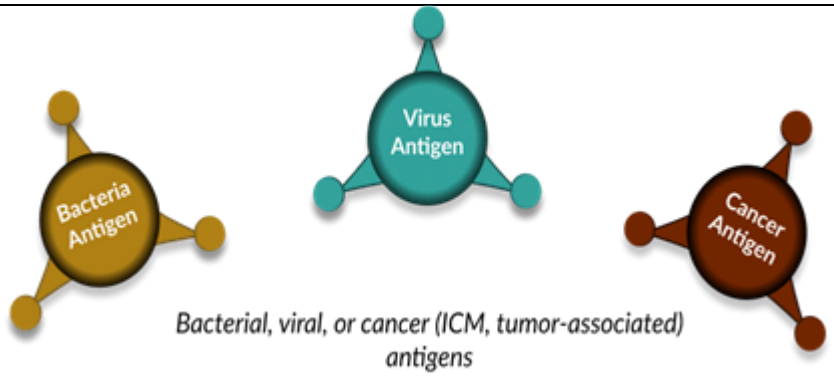

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Figure 17. Typical examples of antigens

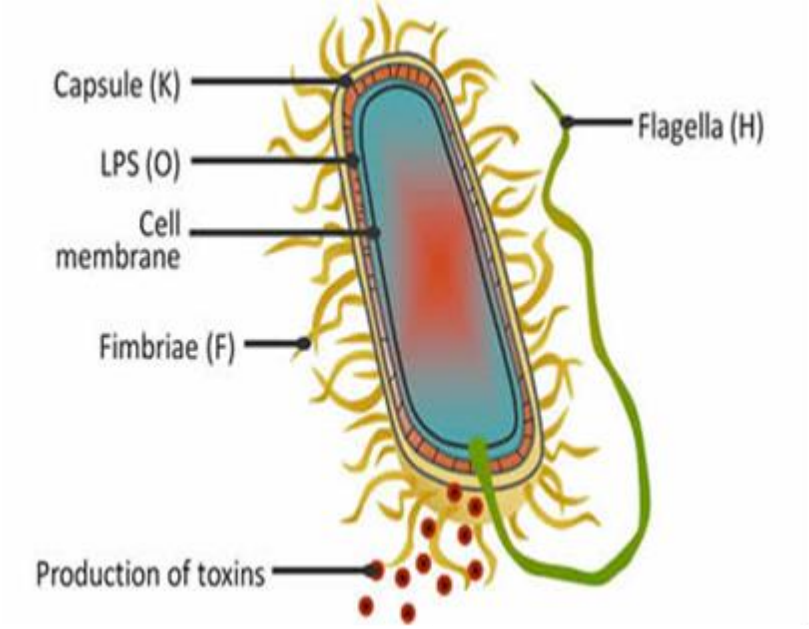

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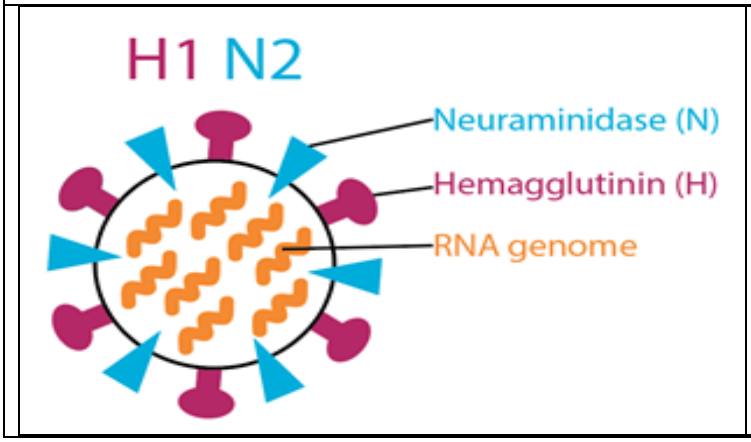
Figure 18. Antigenic structure of bacterial cell



Has two types of spikes (peplomers):

Hemagglutinin (H):

4 subtypes have been found in human isolates (H 1-3, 5 from 15).

Neuraminidase (N):

2 subtypes have been found in human isolates (N 1, 2 from 19)

https://i2.wp.com/sitn.hms.harvard.edu/wp-content/uploads/2018/04/Figure_1_weiner.png

Figure 19. Antigenic structure of influenza virus

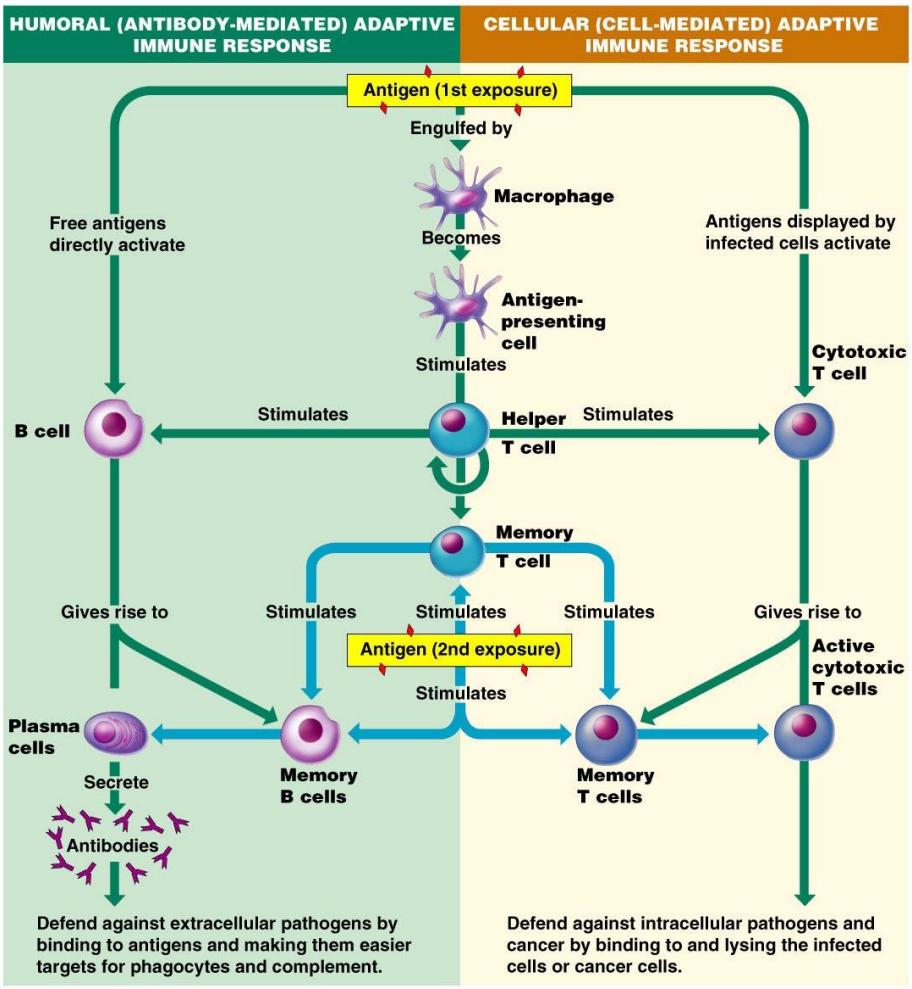
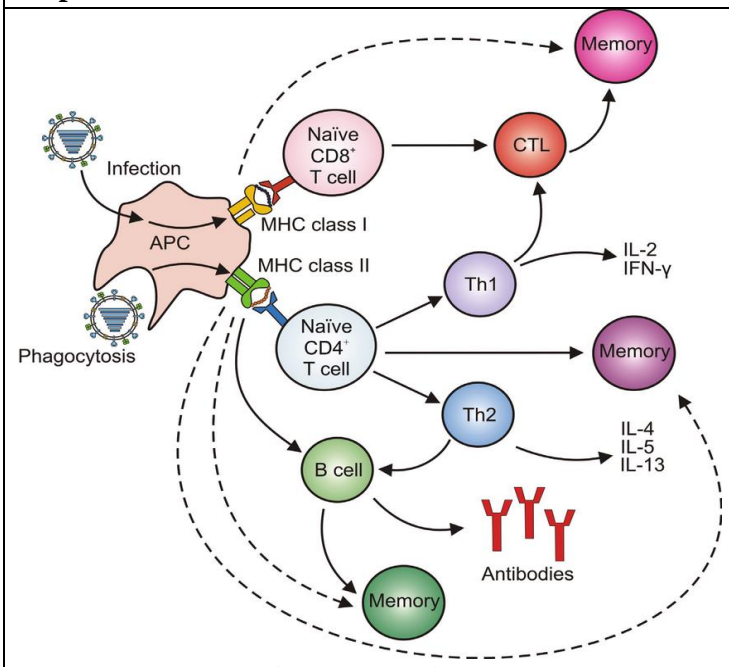
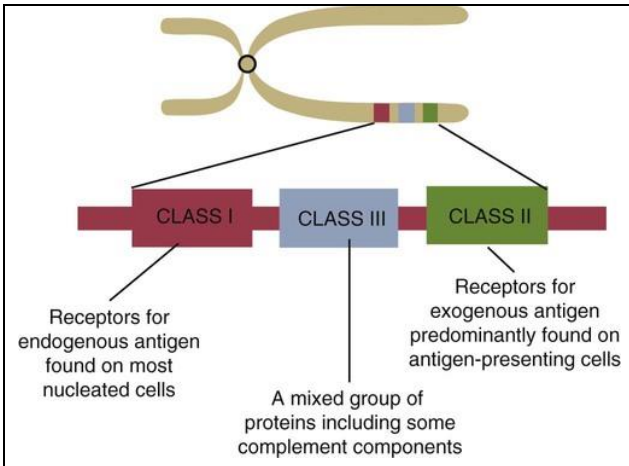


Figure 20. Co-operation between immune cells in the process of forming an adaptive immune response



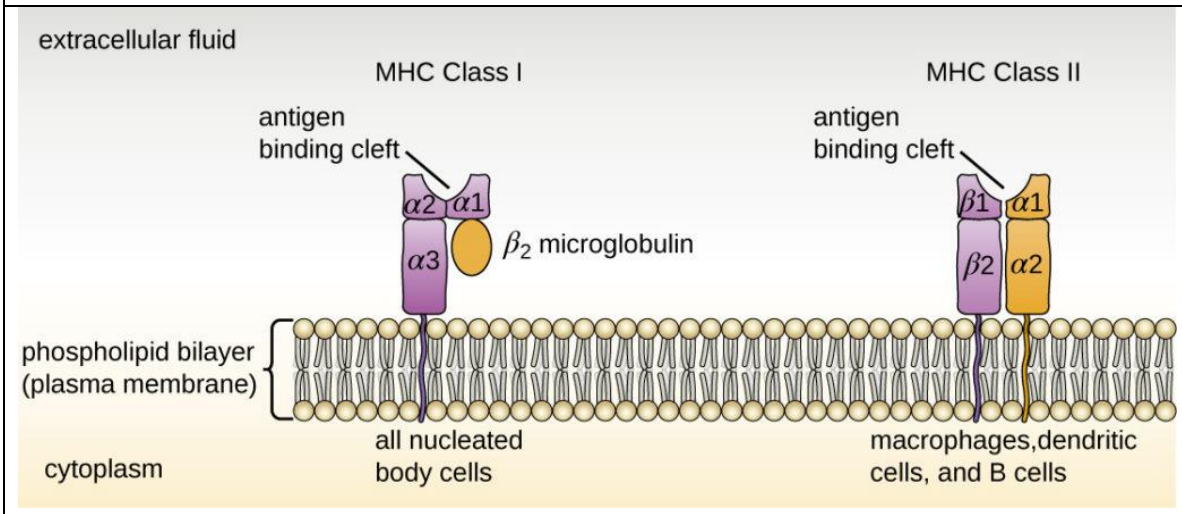
https://www.researchgate.net/figure/Induction-of-humoral-and-cellular-immunity-Induction-of-immune-responses-after-a-primary_fig1_233739031

Figure 21. Stages of immune response formation



https://www.google.com/imgres?imgurl=https%3A%2F%2Fveteriankey.com%2Fwp-content%2Fuploads%2F2016%2F07%2FB9781455703623000112_f011-002-9781455703623.jpg&imgrefurl=https%3A%2F%2Fveteriankey.com%2Fthe-major-histocompatibility-complex%2F&tbnid=Qvf9oe7L5pe2CM&vet=12ahUKEwiivZHHtbDoAhUVTpoKHe9JBK4QMygSegUIARDzAQ..i&docid=n90Nm0jd7jk1oM&w=500&h=375&q=major%20histocompatibility%20complex%20images&ved=2ahUKEwiivZHHtbDoAhUVTpoKHe9JBK4QMygSegUIARDzAQ

Figure 22. Classes of the MHC antigens



https://courses.lumenlearning.com/microbiology/chapter/major-histocompatibility-complexes-and-antigen-presenting-cells/

Figure 23. Structure of MHC

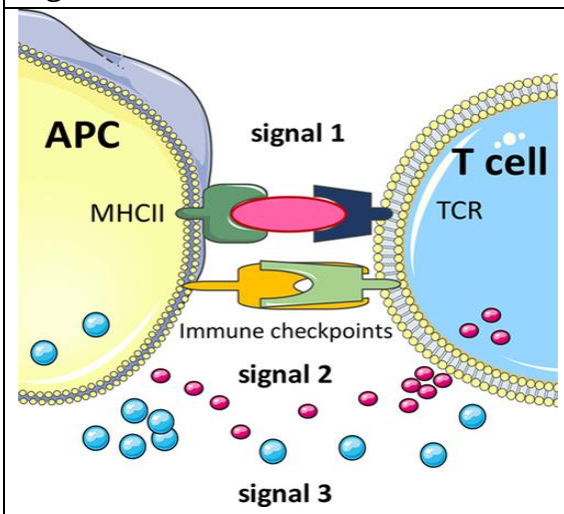


Figure 24. Activation of T cell

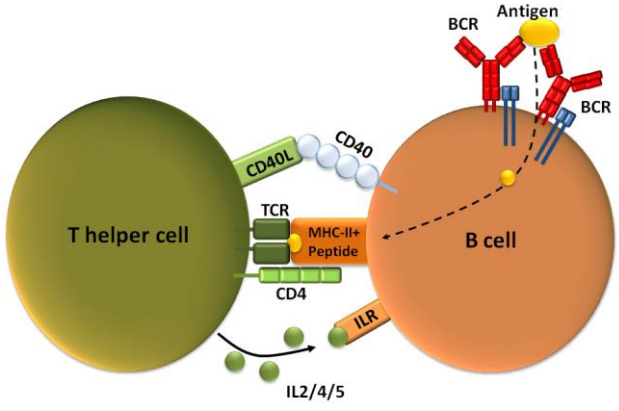
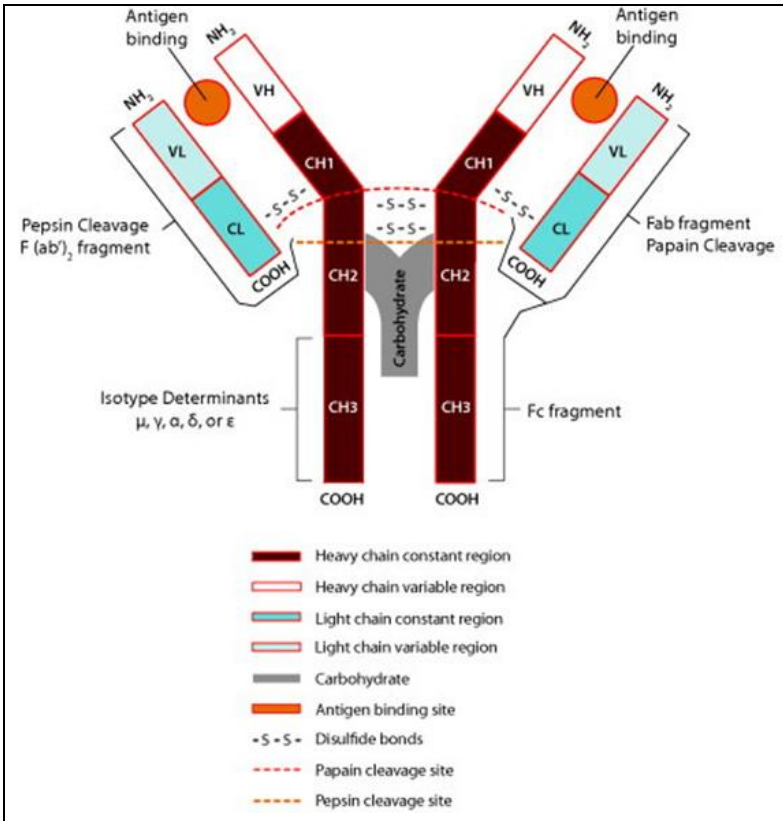
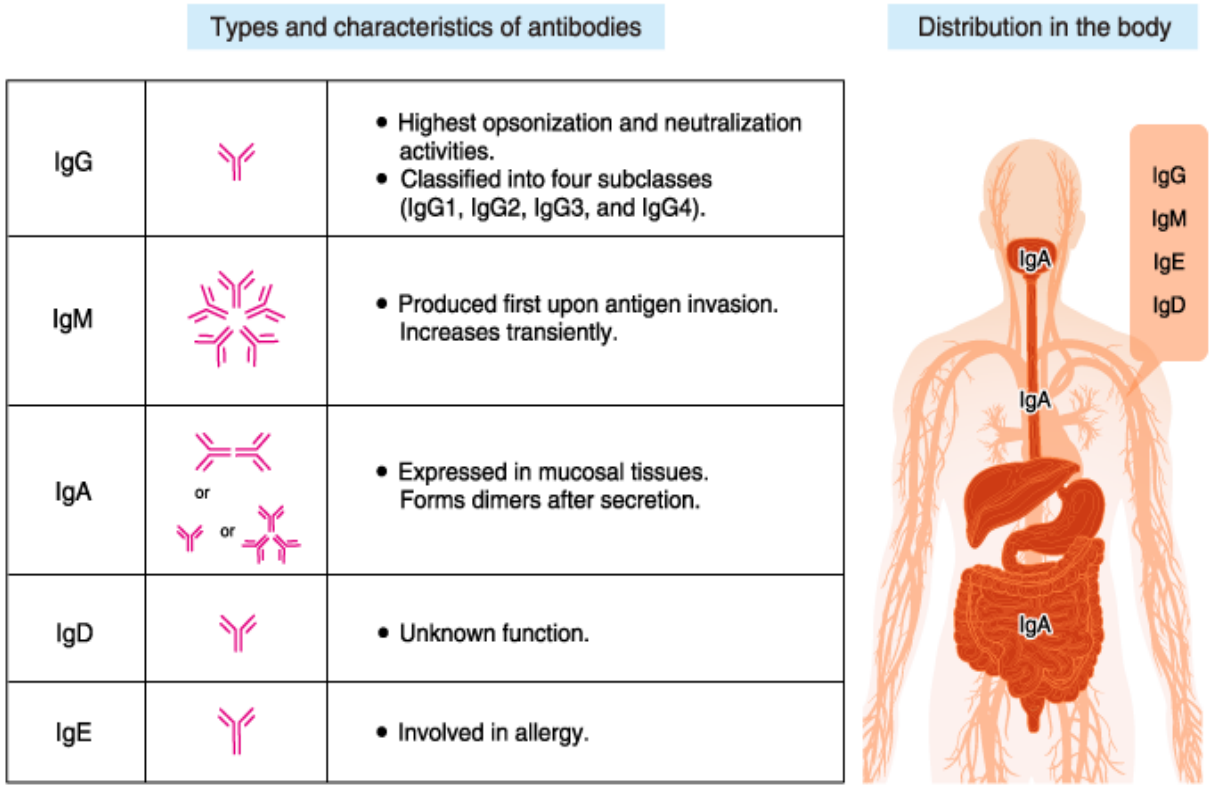


Figure 25. Activation of B cell



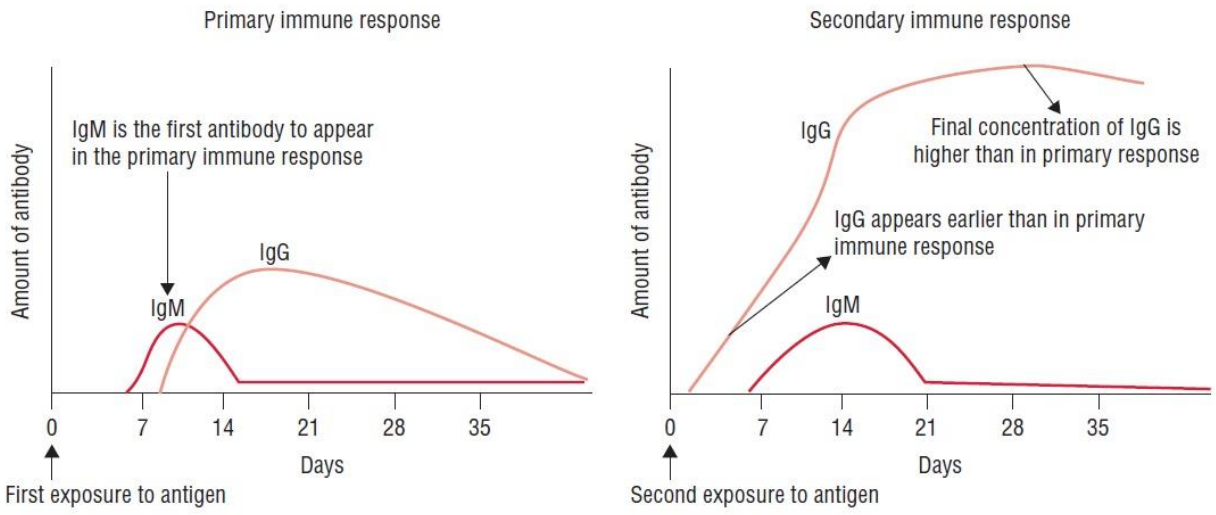
https://www.sigmaaldrich.com/technical-documents/articles/biology/antibody-basics.html

Figure 26. Schematic diagram of an immunoglobulin



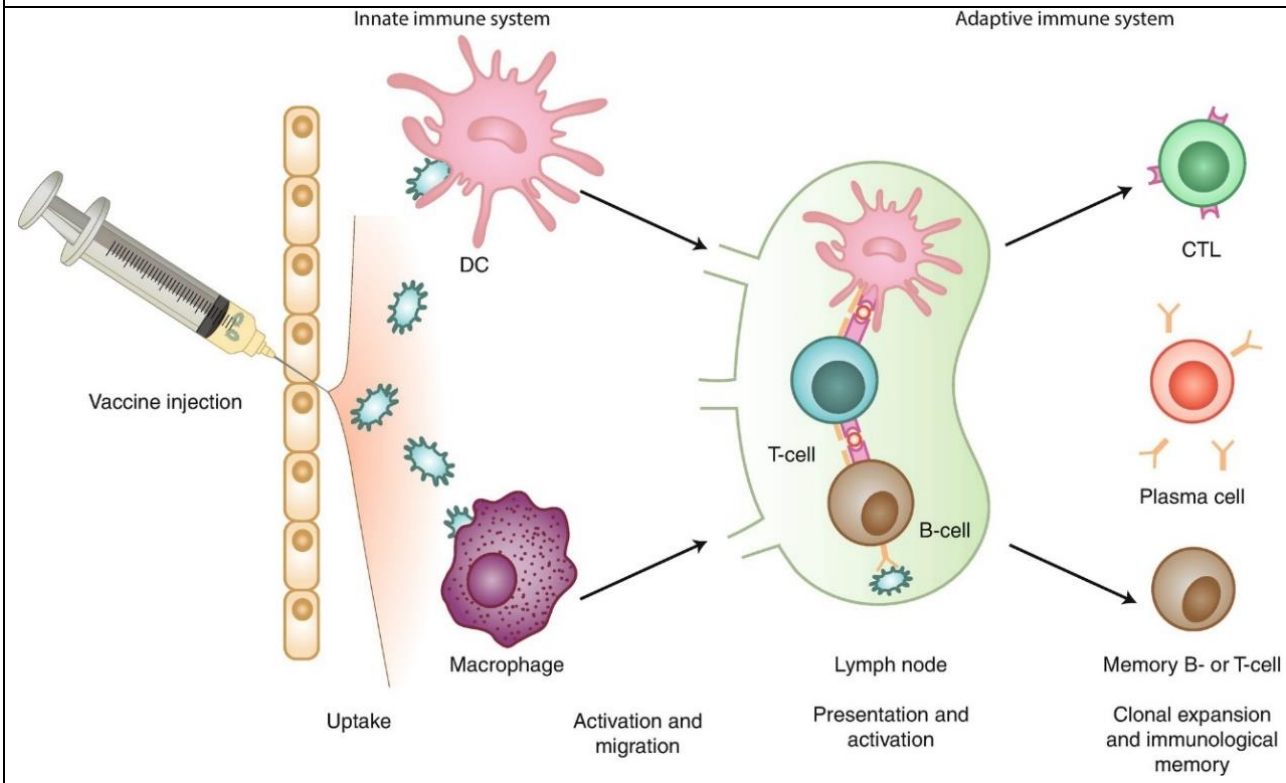
https://ruo.mbl.co.jp/bio/e/support/method/antibody-isotype.html

Figure 27. Classes of the immunoglobulins – characteristics



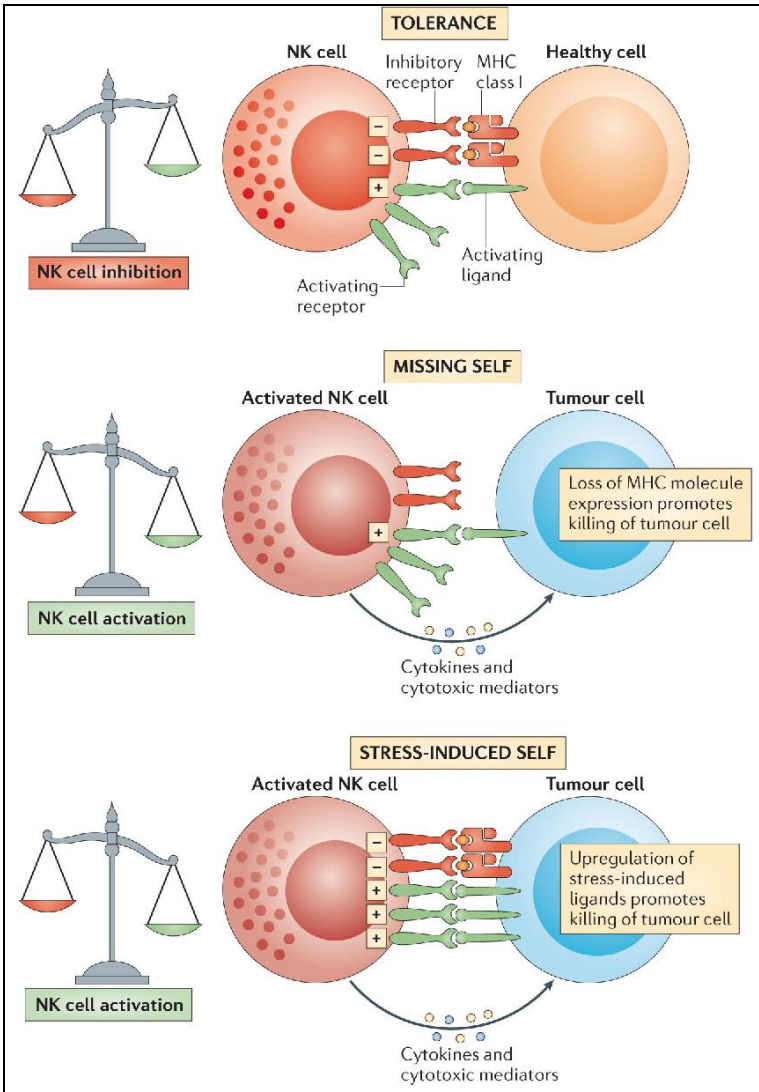
https://microbenotes.com/differences-between-primary-and-secondary-immune-response/

Figure 28. Stages of the immune response



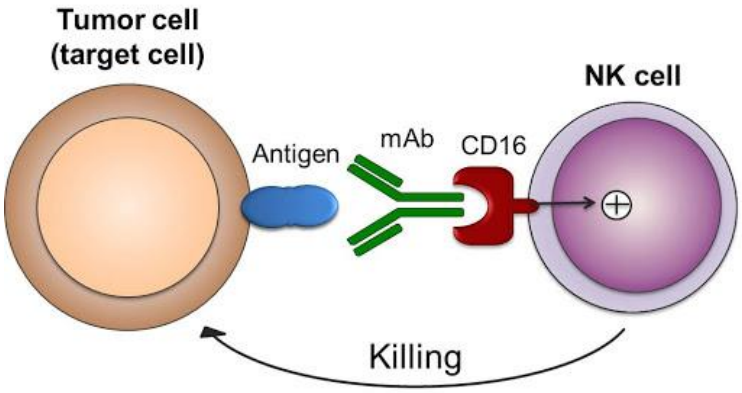
https://media.springernature.com/original/springer-static/image/chp%3A10.1007%2F978-3-030-00710-2_14/MediaObjects/453660_5_En_14_Fig3_HTML.jpg

Figure 29. Immunological memory



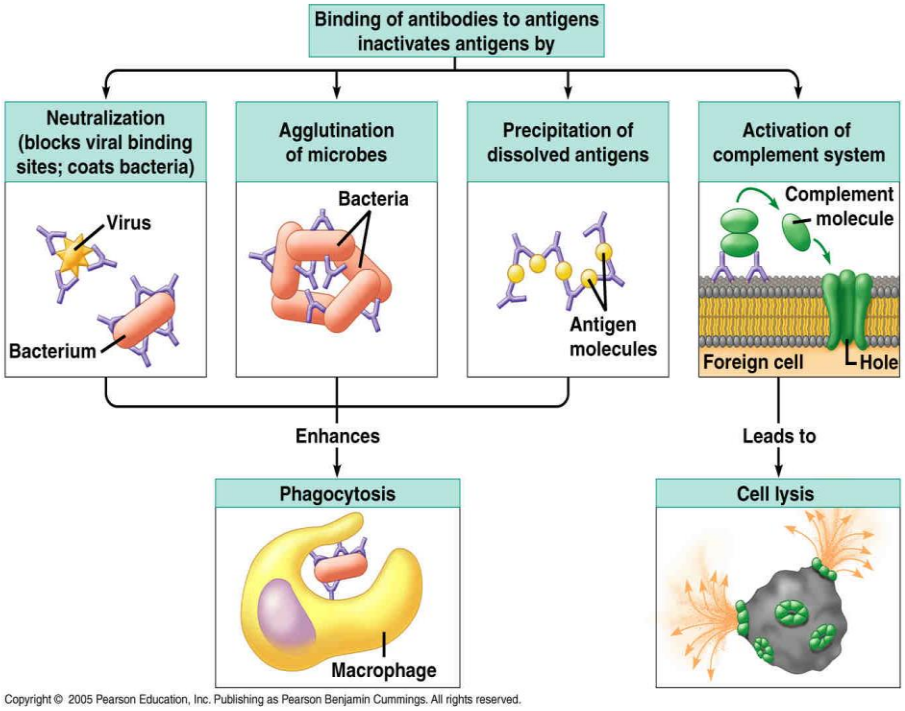
https://www.semanticscholar.org/paper/Targeting-natural-killer-cells-and-natural-killer-T-Vivier-Ugolini/dcf553aaa0061a7361bba9cbee7907ce610a231b/figure/0

Figure 30. First mechanism by detecting the presence of stress signals. Extracellular killing of target cell by NK-cell (infected or tumor cell)



https://www.google.com/imgres?imgurl=http%3A%2F%2Fwww.stream.wum.edu.pl%2Fimages%2FFigure3.jpg&imgrefurl=http%3A%2F%2Fwww.stream.wum.edu.pl%2Fen%2Fknowledge-base%2F96-nk-cells-applications-in-immuno-oncology&tbnid=XOigdZBcYFENkM&vet=12ahUKEwij08G84LXoAhVHbJoKHYjgDn8QMyglegQIARBL..i&docid=9BNXc2Fcw1UAaM&w=1076&h=586&q=normal%20killers%20mechanisms%20images&hl=uk&ved=2ahUKEwij08G84LXoAhVHbJoKHYjgDn8QMyglegQIARBL

Figure 31. The third mechanism with feedback activation of NK cell by specific antibody



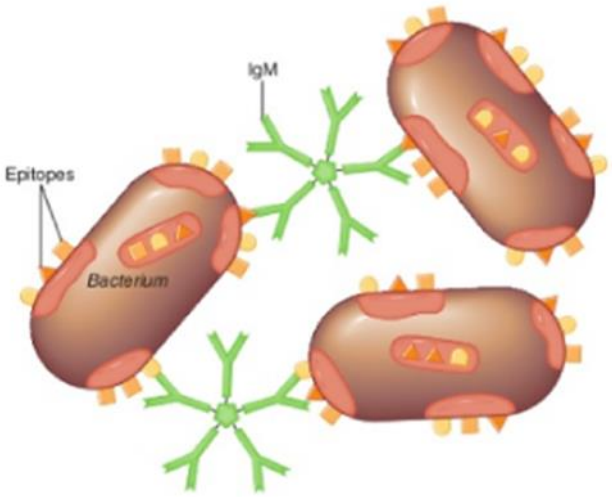
https://www.austincc.edu/apreview/EmphasisItems/Inflammatoryresponse.html

Figure 32. Effects of binding antibodies to antigens

Characteristics	Type I	Type II	Type III	Type IV
Immune reaction	IgE (soluble antigen)	IgG/IgM (cell-associated antigen)	IgG/IgM (soluble antigen)	T cells (Soluble or cell-bound antigen)
Description	Immediate hypersensitivity	Antibody-mediated cytotoxic hypersensitivity	Immune complex-mediated hypersensitivity	Cell-mediated hypersensitivity
Reaction time	5-30 minutes	Minutes - hours	3 – 8 hours	48 – 72 hours
Effector mechanism	Mast cell activation	Complement mediated phagocytosis	Tissue damage induced by immune complexes	T cell mediated inflammation or cytotoxicity
Clinical manifestation	Allergic rhinitis Asthma Anaphylaxis	Drug allergy Graves disease Anemia	Rheumatoid arthritis Serum sickness SLE	Contact dermatitis Graft rejection Chronic asthma

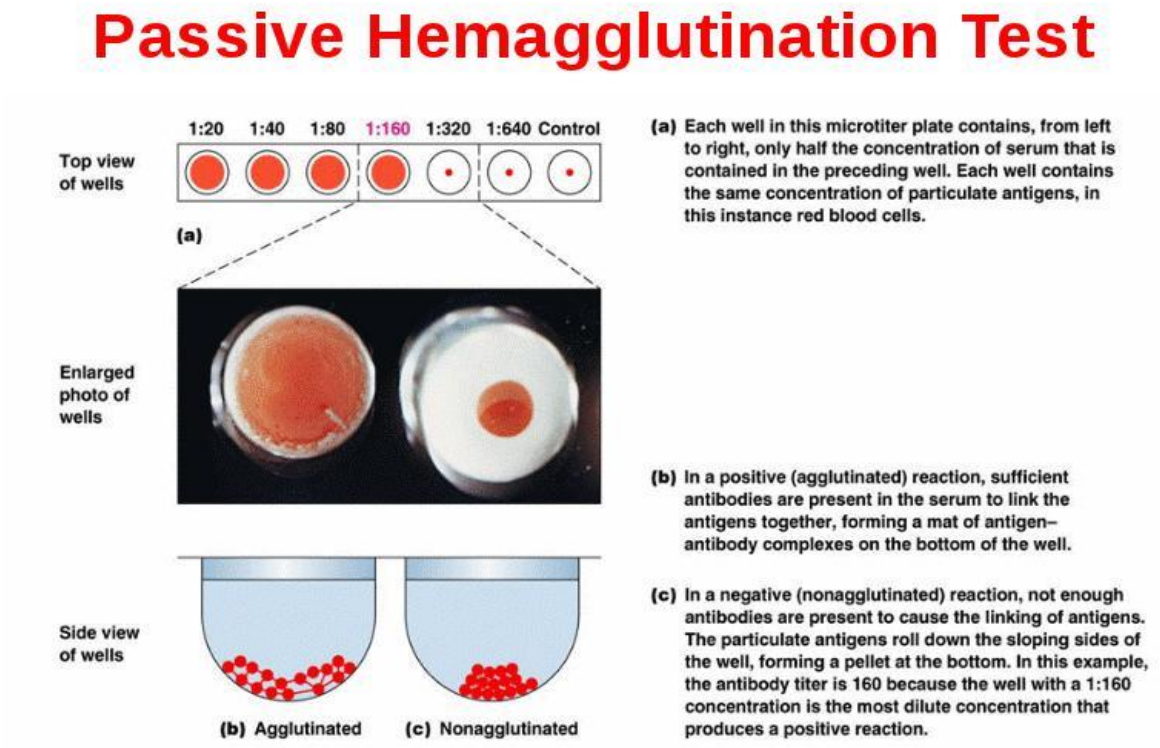
http://gbsleiden.com/allergy/

Figure 33. Types of allergic reactions



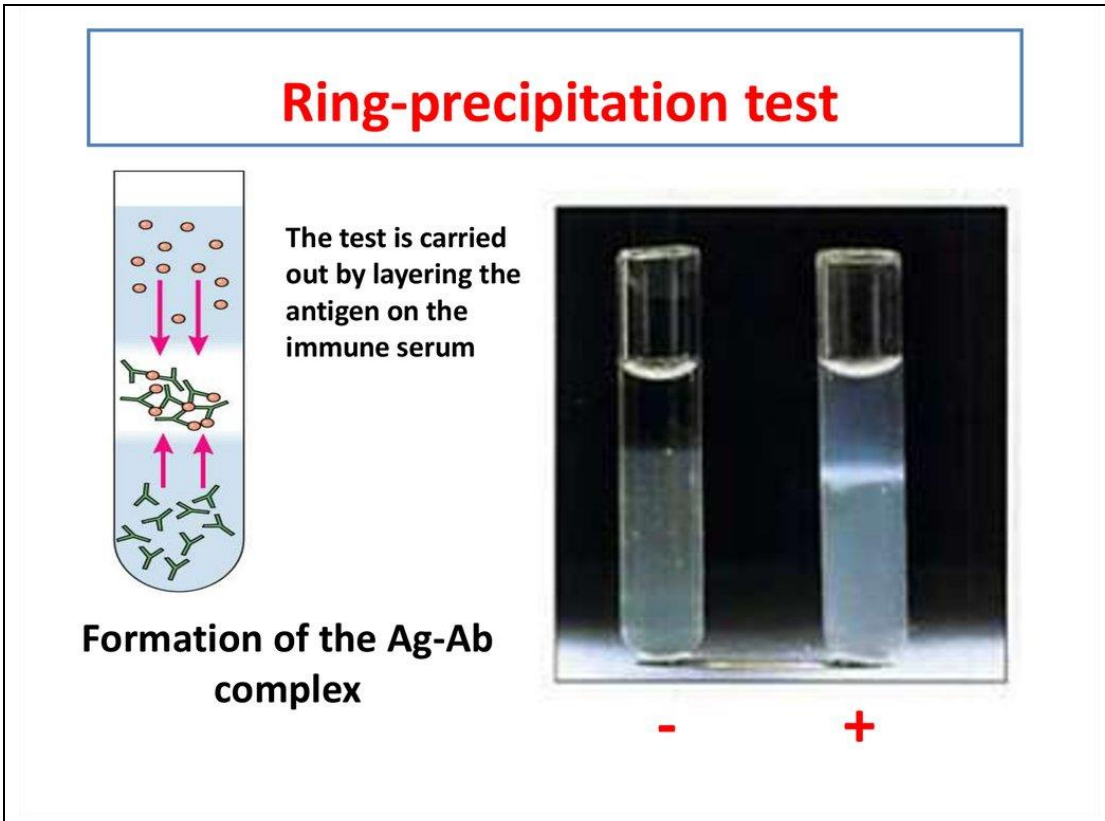
https://www.slideserve.com/leone/antigen-antibody-reactions

Figure 34. Agglutination phenomenon



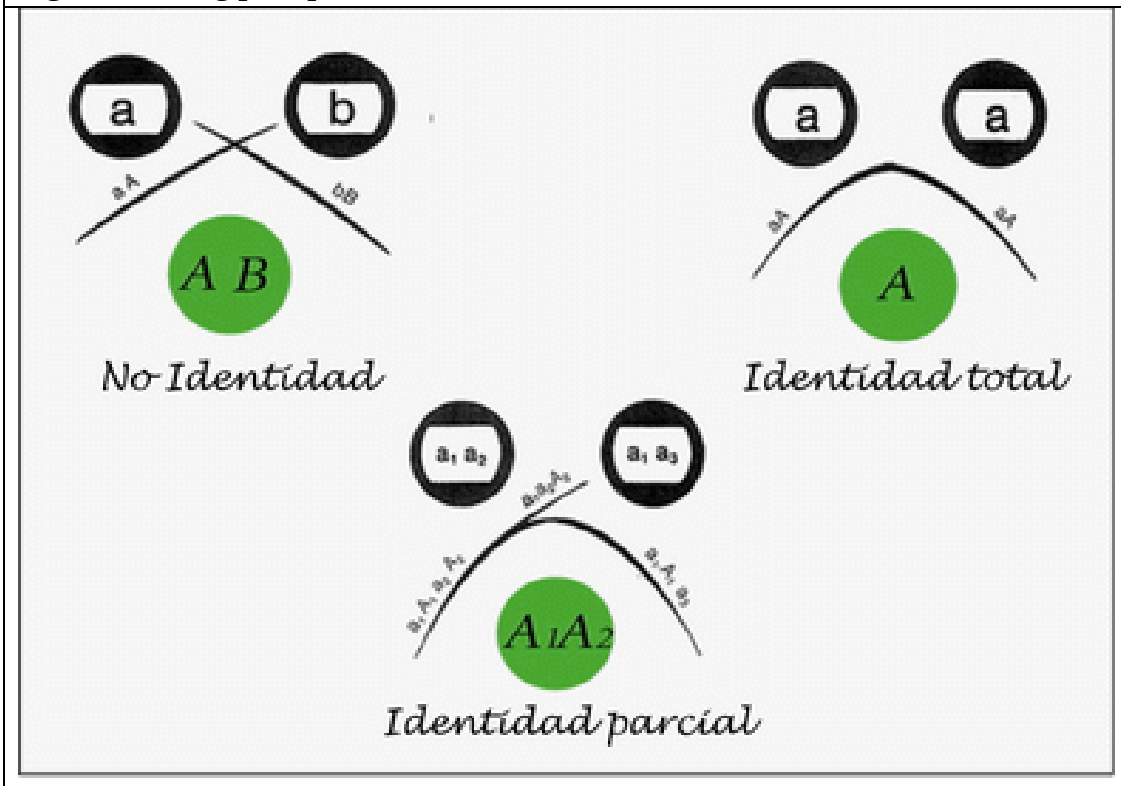
https://presentacii.ru/presentation/Agglutination-and-Precipitation-Reactions

Figure 35. Indirect (passive) hemagglutination test



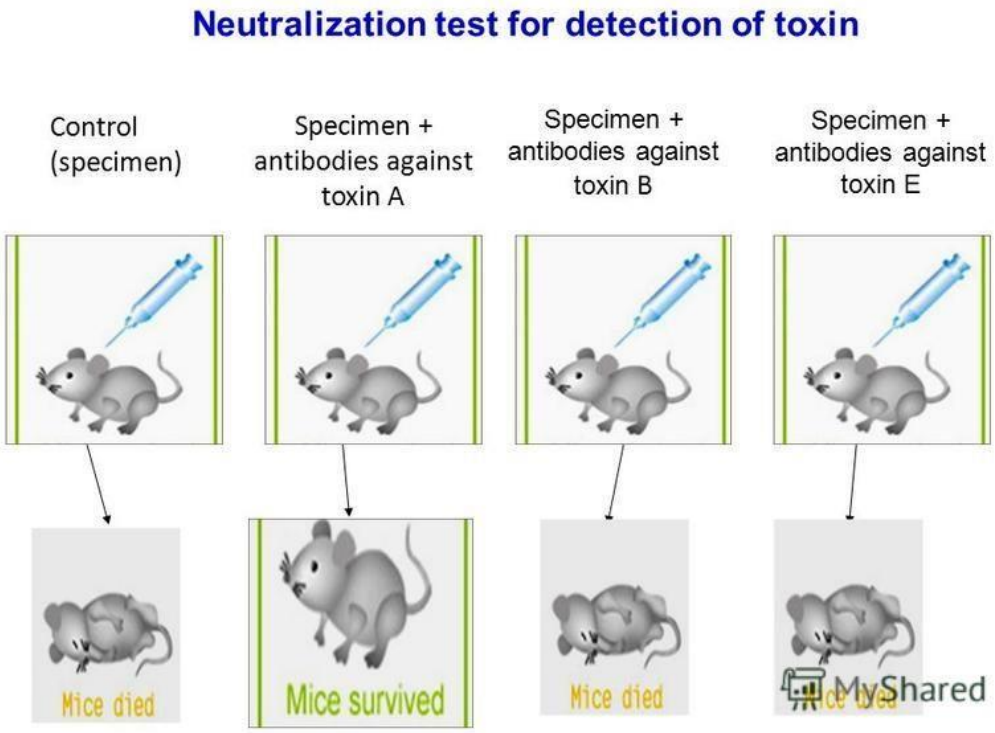
https://en.ppt-online.org/298179

Figure 36. Ring precipitation test



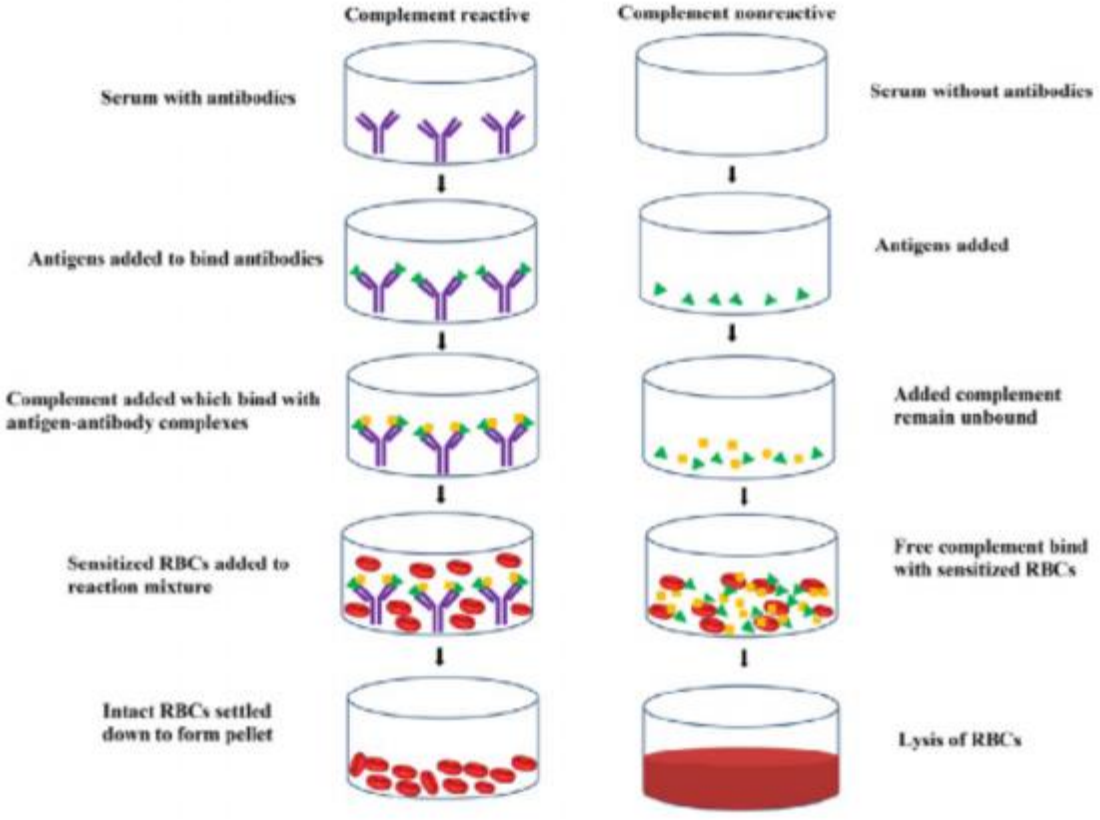
https://www.monografias.com/trabajos911/interaccion-antigeno-anticuerpo/interaccion-antigeno-anticuerpo.shtml

Figure 37. Ouchterlony's double immunodiffusion test relatedness of antigen



http://www.myshared.ru/slide/1387790/

Figure 38. Toxin-antitoxin neutralization is measured in vivo



https://www.researchgate.net/figure/Principle-of-Complement-fixation-test_fig9_291171327

Figure 39. Complement-Fixation Test

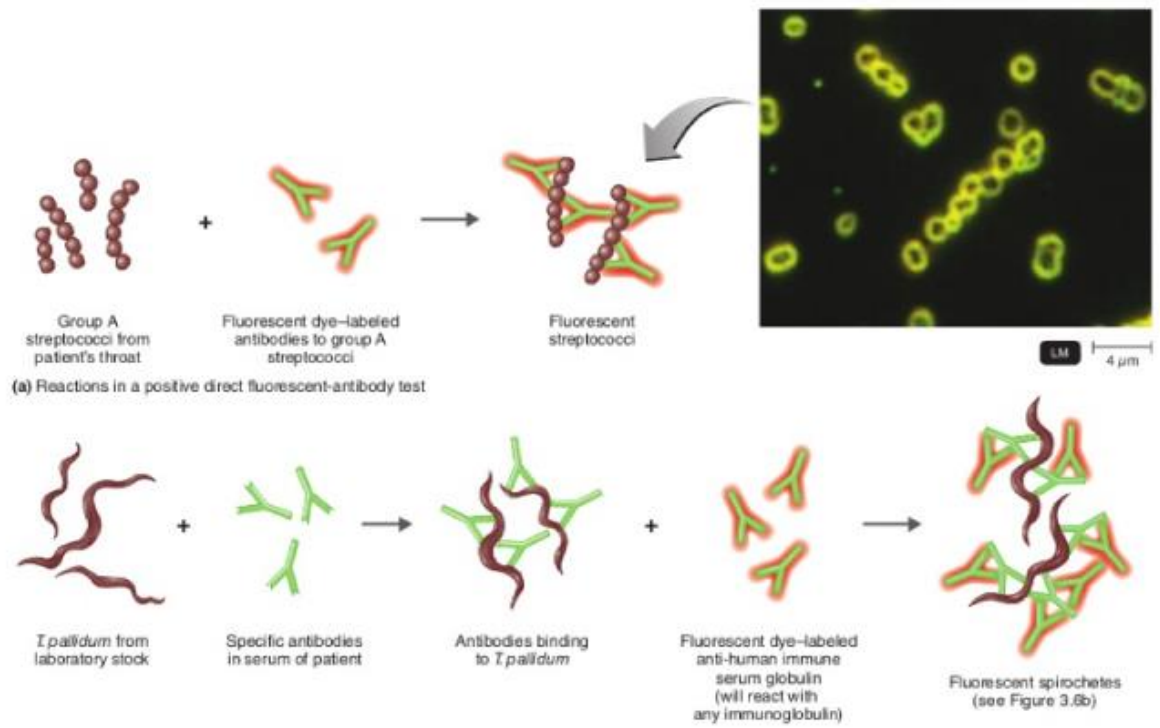
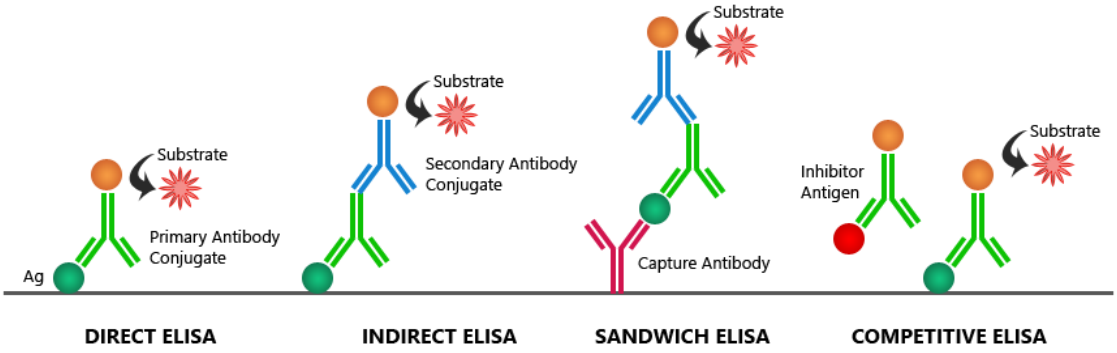
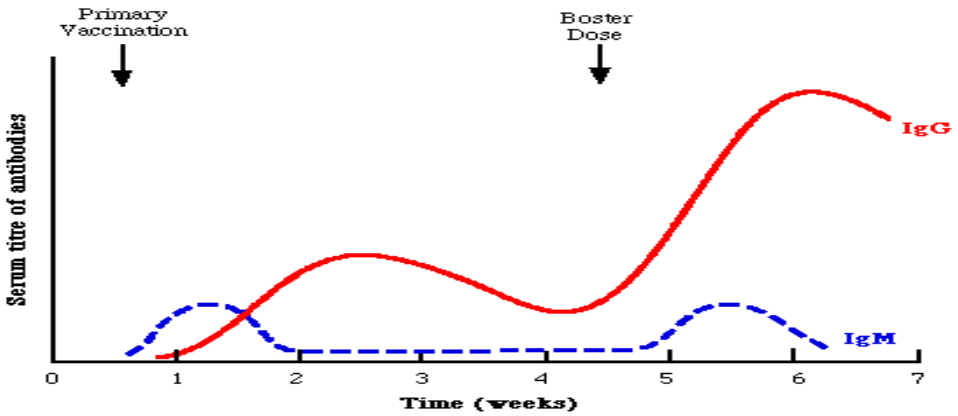


Figure 40. Direct and Indirect immunofluorescence tests



https://www.bosterbio.com/protocol-and-troubleshooting/elisa-principle

Figure 41. Four most common ELISA **types**



https://aces.nmsu.edu/pubs/_b/B222/images/fig1.jpg

Figure 42. Mechanism of vaccines action

Посібник для практичних занять з мікробіології, вірусології та імунології для аудиторної та позааудиторної роботи англомовних студентів. Він може бути використаний для підготовки до практичних занять, поточного контролю знань, підсумкового модульного контролю з предмету.

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