Ministry of Health, Republic of Belarus

Institution of Education

“Grodno State Medical University”

Department of Microbiology, Virology and Immunology named after S.I.Gelberg

IMMUNOLOGY

Training appliance for students of the Department for International Students

IMMUNOLOGY AS A SCIENCE. INNATE IMMUNIY

Theme № 10

IMMUNOLOGY AS A SCIENCE

- **Immunology is a bio-medical science studying different aspects of immunity.**

Growing importance of immunology for all branches of medicine in modern time is based on the next facts:

- appearance of new infections which cause debilitation of human organism,
- accumulation of the genotypes in human population encoding immune deficiency,
- increase of the cases of allergy,
- uncontrolled and wide use of immune stimulators,
- complications of vaccination.

The statement of immunity (what immunity is)

The mechanism of defence of macro-organism against genetically alien agents – ***antigens*** **.**

Antigens are substances (food, drugs, etc) or cells (microbes or graft cells) entering the human organism from outsides or own but not useful cells.

Immunity includes:

the ability to recognise and to destroy the antigens.

CHARACTERISTICS OF FACTORS AND MECHANISMS OF DEFENCE OF THE HUMAN ORGANISM AGAINST INFECTIOUS AGENTS

The scheme of defence of human organism against antigens

The human defence systems divided into two groups:

1. Constitutive host defence - non-specific resistance
   - innate immunity
2. Inducible host defence - immunological reactivity: adaptive/specific immunity
   - acquired immunity

INNATE IMMUNITY

The scheme of defence of human organism against antigens: innate immunity

Innate immunity (non - specific resistance)

- present in human organism from birth
- characterized as non-specific: acts on many microorganisms
- does not become more efficient on subsequent exposure to the same microorganisms

Innate immunity: non-immune factors

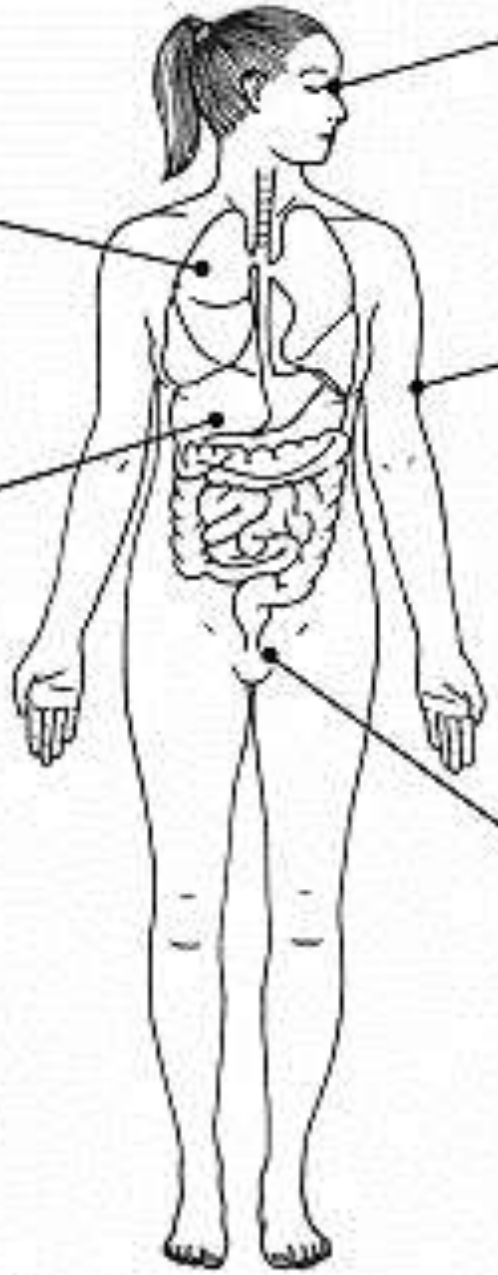
Non-immune factors of innate immunity:

1. Species resistance: absence of specific tissue or cellular receptors for attachment (colonization) by the pathogen, lack of a target site for a microbial toxin, etc.
2. Individual resistance: age, stress, diet, sex, race, etc.
3. Anatomical defences: natural barriers provided by skin and mucous membranes.
4. Bactericidal effect of secretions of sweat, sebaceous, salivary glands and glands of stomach.

Innate immunity: non-immune factors

Non-immune factors of innate immunity:

5. Microbial antagonism: competition of normal microflora with non-indigenous species for binding (colonization) sites.
6. Specific antagonism of normal microflora against non-indigenous species and regulation of the composition of intestinal microbiocenosis : production of bacteriocins.
7. Production of antimicrobial substances by human organism that inhibit pathogenic microorganisms: fatty acids and peroxides.



Eyes
1. Washing by tears
2. Lysozyme

Respiratory tract
1. Mucus
2. Cillated epithelium
3. Antibody
4. Phagocytosis

Skin
1. Anatomic barrier; sweat, sebum
2. Antimicrobial secretions; lactic acid, free fatty acids
3. Low pH (GS)
4. Commensal microbes

Digestive tract
1. Stomach acidity
2. Normal flora
3. Intestine-Alkaline pH
4. Mechanical flushing
5. Enzymes
   eg. Lysozyme
6. Bacteriocins (colon)

Genitourinary tract
1. Washing by urine
2. Acidity of urine
3. Lysozyme
4. Vaginal lactic acid

The scheme of defence of human organism against alien agents (antigens)

- **Innate immunity**

Immune factors

Humoral

1. **Complement**
2. **Properdin**
3. **Lysozyme**
4. **β-lysines**
5. **Fibronectin**
6. **Acute phase proteins**
7. **Interferons**

Cellular

1. **Phagocytes**
2. **NK-cells**

ANTIMICROBIAL SUBSTANCES OF HOST ORIGIN PRESENT IN BODY FLUIDS AND ORGANIZED TISSUES

Substance	Common Sources	Chemical Composition	Activity
Lysozyme	Serum, saliva, sweat, tears, - all physiological liquids excluding liquor and front chamber of eye. Produced by phagocytes.	Protein	Bacterial cell lysis: destroys cell wall of bacteria, activates phagocytes
Complement	Serum	Protein-carbohydrate lipoprotein complex	Cell death or lysis of bacteria; participates in inflammation
Basic proteins and polypeptides (ß-lysins, defensins, etc)	Serum or organized tissues. Produced by trombocytes, neutrophyls and epithelial cells	Proteins or basic peptides	Disruption of bacterial plasma membrane, killing of bacteria

ANTIMICROBIAL SUBSTANCES OF HOST ORIGIN PRESENT IN BODY FLUIDS AND ORGANIZED TISSUES

Substance	Common Sources	Chemical Composition	Activity
Fibronectin	Serum and mucosal surfaces. Produced by macrophages.	Glycoprotein	Clearance of bacteria: removing of bacteria from macroorganism, blocks bacterial adhesion.
Interferons	Virus-infected cells, lymphocytes	Low molecular weight protein	Resistance to virus infections , antitumoral effect, promote activation of immune system
Interleukins	Macrophages, lymphocytes	Protein	Cause fever; promote activation of immune system
Peroxidase	Saliva, tissues, cells (neutrophils)	Protein	Act with peroxide to cause lethal oxidations of cells

ANTIMICROBIAL SUBSTANCES OF HOST ORIGIN PRESENT IN BODY FLUIDS AND ORGANIZED TISSUES

Substance	Common Sources	Chemical Composition	Activity
Acute phase proteins: C-reactive protein (CRP) and other proteins (pentraksin, surfactant)	CRP produced in liver, surfactant - surface active substance in lungs	Proteins	Decrease the virulence of infectious agent, activate complement, promote activation of immune system
Properdin	Serum	Protein	Activates the complement in alternative pathway
Lactoferrin and transferrin	Body secretions, serum, organized tissue spaces	Glycoprotein	Inhibit microbial growth by binding (withholding) iron

Inductors of synthesis of interferones

- proteins
- ds RNA
- bacteria
- viruses
- products of bacterial degradation
- some synthetic polymers (polyanions)

INTERFERONS

Type	Cellular origin	Predominant effect
α-IFN	Leukocytes	•an antiviral •antitumoral
β- IFN	Fibroblasts	•antitumoral
γ- IFN	Lymphocytes	•immunomodulating

Factors of nonspecific resistance (innate immunity)

NK-CELLS

- Large lymphocytes having their origin from the population of zero lymphocytes.
  - Participate in **<u>extracellular killing</u>** of tumoral cells and the cells presenting alien antigen on their surface
    - they act by the way of perforin excreting (their effect is analogical to MAC of the compliment system).

THE COMPLEMENT SYSTEM

The complement system (main characteristics)

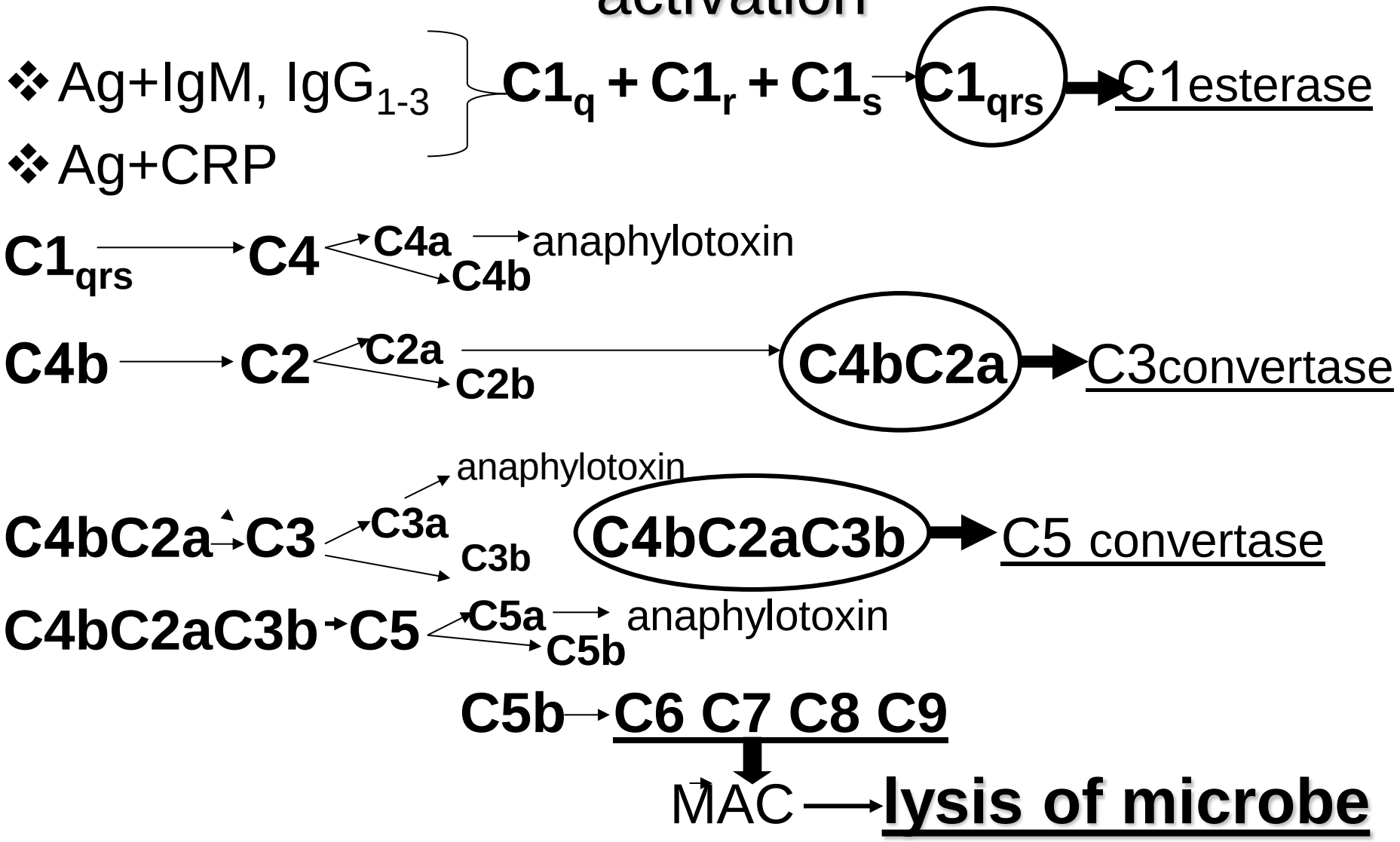
- Multi-enzymatic system of serum proteins made up of 9 major components (C1 - C9)
- Contains 20 proteins – called fractions of the complement
- Is activated by the way of cascade reaction process when the product of previous reaction initiates the following reaction

Classical pathway of the complement activation

ACTIVATORS

- Ag+IgM,
- Ag+IgG_{1-3}
- Ag+CRP

Classical pathway of the complement activation



Stages of activation of complement by the classical pathway

Complement proteins in the blood are not activated until they interact with immune complexes. Immunoglobulins (IgG and IgM) can "fix complement". This initiates a "cascade reaction":

(1) C1, consisting of subunits C1q, C1r and C1s, binds to Fc of IgM or Fc of two adjacent IgGs. C1 is activated.

(2) Activated C1 cleaves C2----> C2a + C2b and C4----> C4a + C4b.

(3) C2a binds C4b---->C2a4b which attaches to the cell membrane.

(4) C2a4b (known as C3 convertase) attracts and enzymatically cleaves C3---->C3a +C3b.
C3a is a phagocyte chemotactic attractant which can bind to a C3a receptor on mast cells inducing degranulation and localized inflammation. **C3b** can attach to the cell membrane or remain attached to IgG or IgM Fc, which in both cases opsonizes cells for phagocytosis (macrophages and neutrophils have C3b receptors on their surfaces).

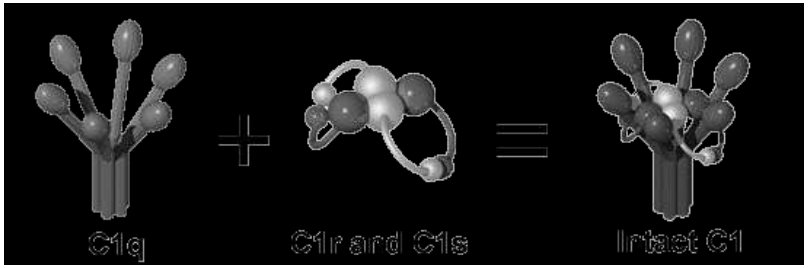
Stages of activation of complement by the classical pathway

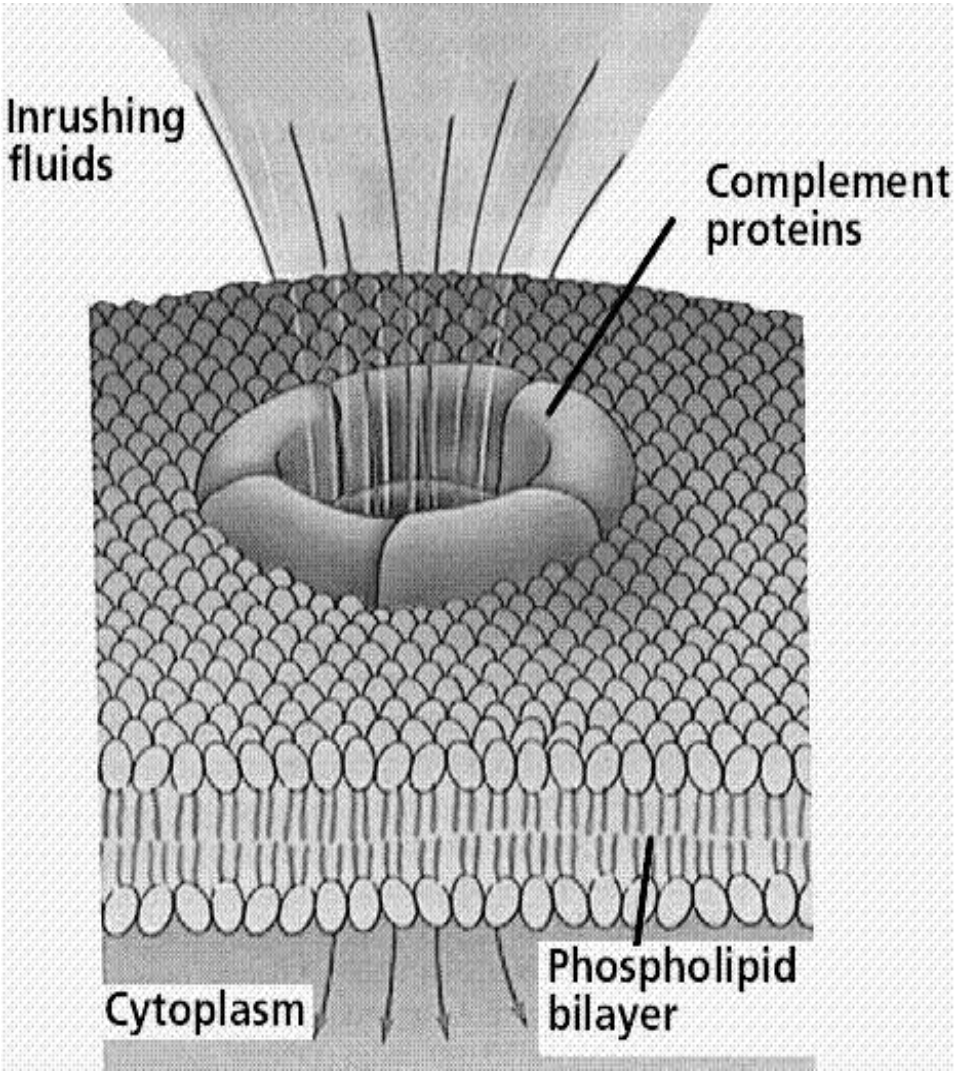
(5) C2a4b + C3b----> C2a4b3b.

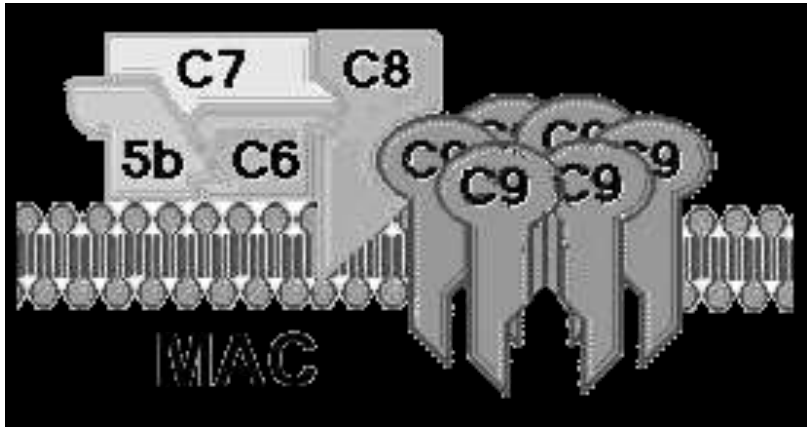
(6) C2a4b3b (C5 convertase) attracts and enzymatically cleaves C5----> C5a + C5b.
C5a is a phagocyte chemotactic attractant and can bind to a C5a receptor on mast cells leading to increased inflammation.

(7) C5b binds C6 and C7----> C5bC6C7 which attaches to the cell surface.

(8) C5bC6C7 attach C8 and C9---->C5bC6C7C8C9. This complex disrupts the cell membrane leading to lysis of the cell, or forms a channel in the outer membrane of Gram-negative bacteria allowing lysozyme access to peptidoglycan leading to lysis.


C1q
C1r and C1s
Intact C1


Inrushing fluids
Complement proteins
Cytoplasm
Phospholipid bilayer


C7
C8
5b
C6
C9
C9
MAC

Alternative pathway of the complement activation

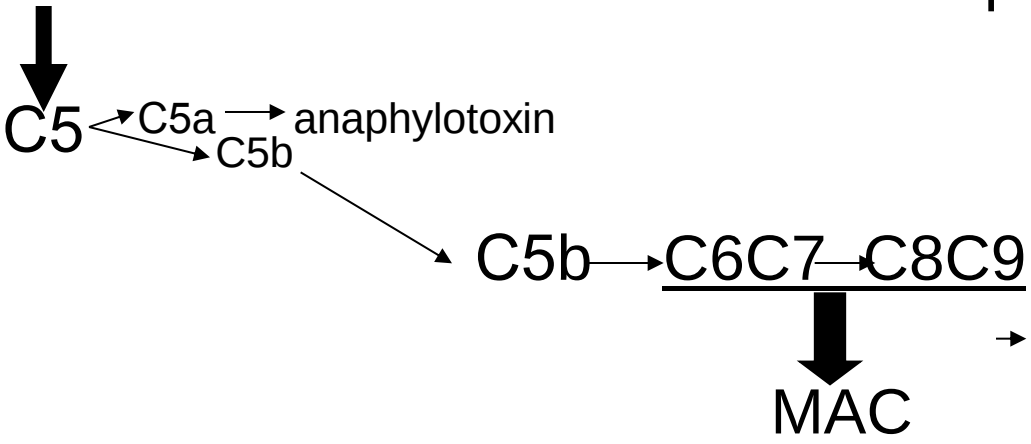
ACTIVATORS

- Endotoxin
- Components of bacterial cell wall: microbial surface sugars and polysaccharides
- Aggregated IgA and IgE

Alternative pathway of the complement activation: main scheme

Properdin + Bacterial polysaccharide + C3 --> C3a + C3b C3a-anaphylotoxin

C3bBb – convertase of alternative pathway



Stages of activation of complement by the alternative pathway

- **An alternative pathway (sometimes called the "properdin pathway") of complement activation is independent of immunoglobulins.**
- **Activators can activate C3 in the absence of C1, C2 or C4.**
- **Properdin combines with bacterial polysaccharides (or certain aggregated antibodies) to activate C3 directly:**

Properdin + Bacterial polysaccharide + C3 ----> C3a + C3b.

C3b + B + proteinase D ----> cleavage of component B into Ba and Bb

fractions.

Bb binds to C3b ----> C3bBb – C5 convertase of alternative pathway

that enzymatically cleaves C5 into **C5a and C5b**.

C6, C7, C8 and C9 continue to react in the same manner as in the classical pathway.

Functions of the complement system

- ❖ Inactivation of microorganisms - when C8 and C9 are bound to the complex, MAC is formed that destroys the membrane of Ag-bearing alien cells (microbes, etc).
- ❖ Activation of phagocytosis:

1. **Opsonisation** - C3b component attaches to C3b receptors on phagocytes and promotes opsonization of the cells: activates engulfment of the alien cells (antigens) by phagocyte.
2. **Chemotaxis** - chemotactic factors C3a and C5a attract phagocytes to the site of infection.
3. **Activation of the process of digestion** - disintegration of engulfed alien cells inside the activated phagocyte.

- ❖ Activation of inflammation – generation of inflammatory factors, C3a and C5a, which focus antimicrobial serum factors and leukocytes into the site of infection.

PHAGOCYTOSIS

Phagocytosis: definition of the term

- Intracellular cytotoxicity: intracellular killing of microbes and inactivation of the alien particles (antigens) which have diameter of 0,1 μm.

The phases of the process of phagocytosis

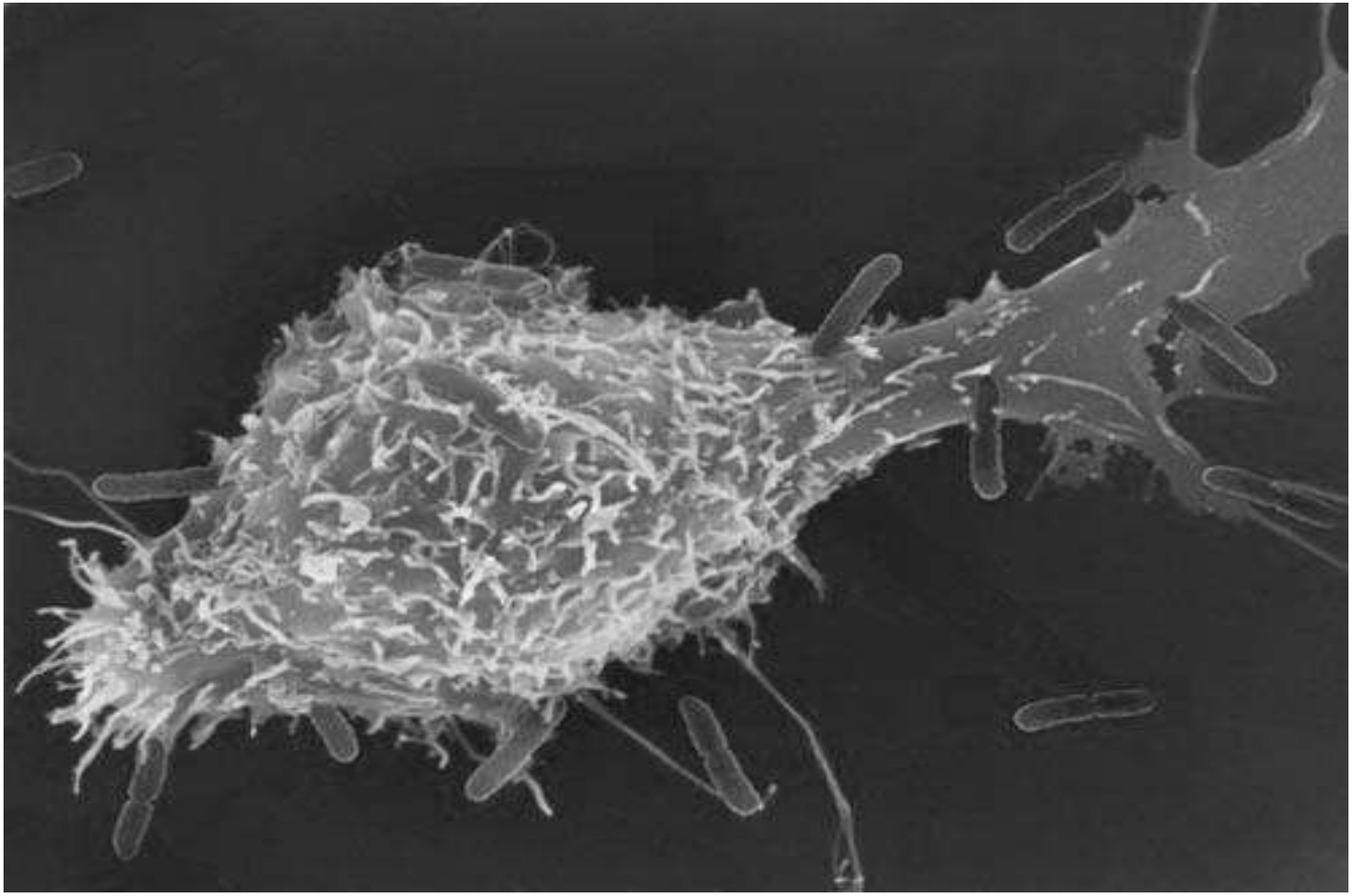
1. Chemotaxis
2. Adhesion
3. Ingestion - engulfment
4. Biodegradation (inactivation)

1 phase of phagocytosis - chemotaxis

- Delivery of phagocytes

  (more frequently they are presented by macrophages) to the object which plays a role of attractant (antigens)

Phagocyte with attached bacteria



2nd phase of phagocytosis – adhesion

Could occur involving 2 different mechanisms:

1. Immunogenic
2. Non - immunogenic

2nd phase of phagocytosis – adhesion: nonimmunogenic phagocytosis

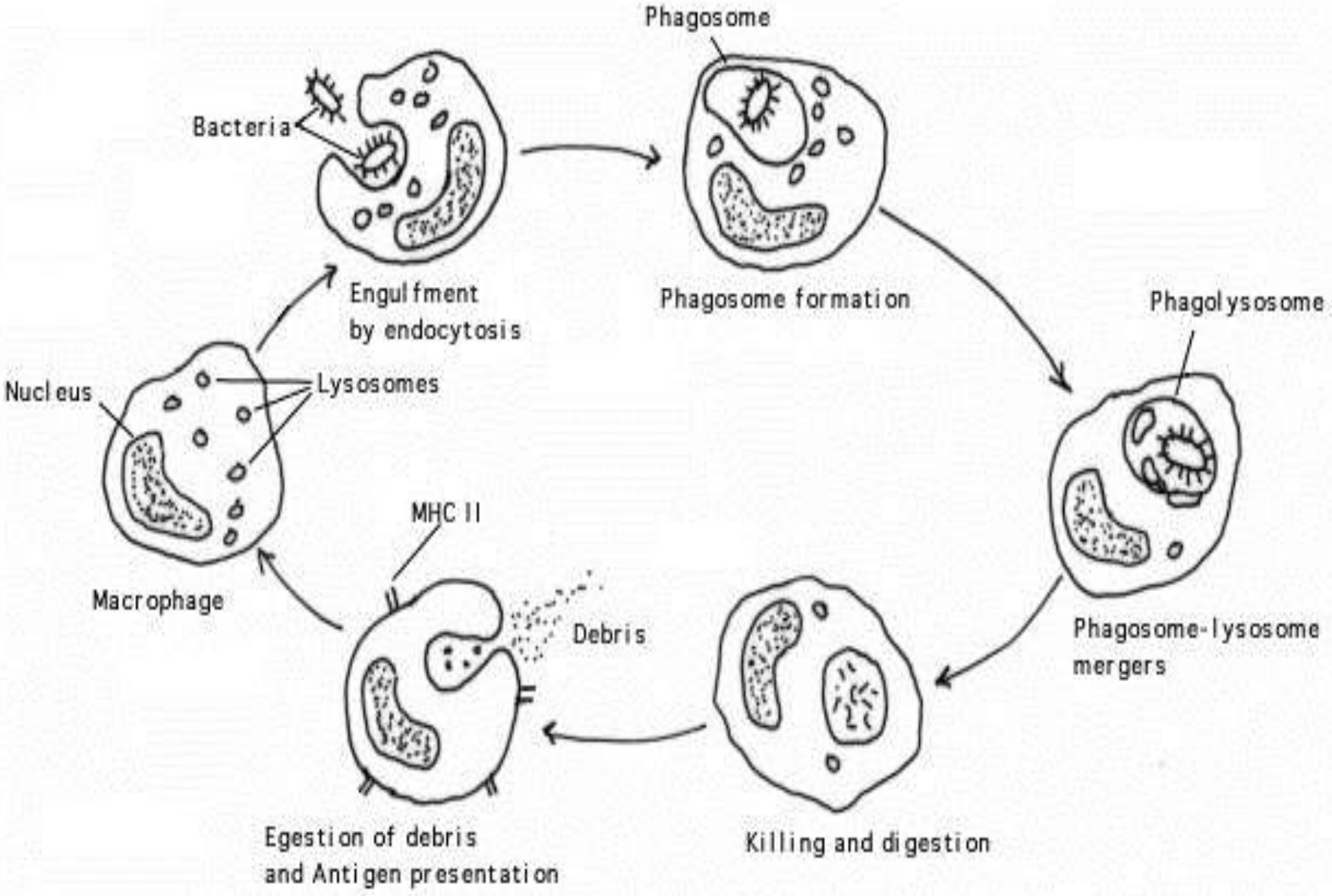
- occurs involving nonspecific adsorption of antigen on the surface of macrophage (or other phagocyte)

2nd phase of phagocytosis – adhesion: immunogenic phagocytosis

- **Involves receptors of macrophages to Fc- fragments of immunoglobulins.**
  1. **Macrophage (MPH) attaches antibodies which located on its surface and participate in attachment of macrophage to the target antigenic cell.**
  2. **Using Fc-receptors MPH binds Fc-fragments of antibodies fixed on the surface of bacteria.**

3rd phase of phagocytosis – ingestion (engulfment of the target)

1. Invagination of the membrane of phagocyte takes place.
2. The target cell (bacteria or other alien cells) is surrounding by large pseudopodia of macrophage enclosing the target cell and ultimately releasing it into the cytoplasm of the phagocyte within a membrane vesicle.
3. Phagosome is forming.
4. Phagosome merges with lysosome forming phagolysosome.


Phagosome
Bacteria
Engulfment
by endocytosis
Phagosome formation
Phagolysosome
Nucleus
Lysosomes
MHC II
Macrophage
Debris
Phagosome-lysosome
mergers
Egestion of debris
and Antigen presentation
Killing and digestion

4th phase of phagocytosis – biodegradation (intracellular digestion or killing)

Intracellular killing of microorganism includes:

- **oxygen-dependent activity** - quick activation of oxidative metabolism in phagocyte (process called «respiratory explosion»)
- **oxygen-independent** - lysosomal granules contain a variety of extremely basic proteins (defenzins, phagocytins) that strongly inhibit bacteria. Activation of enzymes concentrated in lysosome (phospholipase, ribonuclease, etc) occurs which destroy the object of phagocytosis (antigen)

Types of phagocytosis

1. Completed (includes all 4 phases)
2. Noncompleted
   - microbe survives
   - partial degradation of microbial antigen (Ag) occurs that is necessary for the antigen presentation to lymphocytes to activate specific immune response

Functions of phagocytosis (the functions of macrophages)

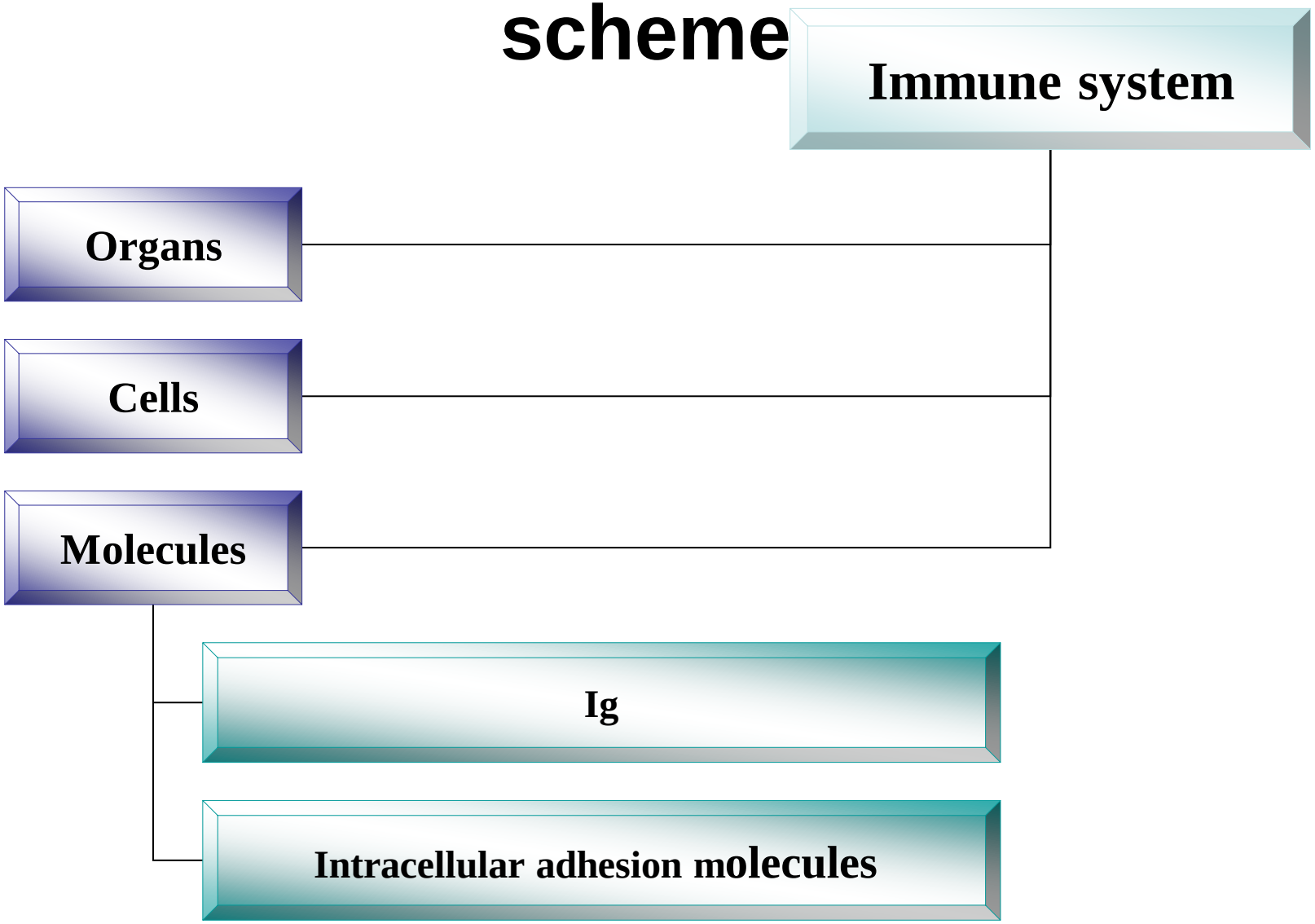
1. Phagocytosis of antigens.
2. Recognition of antigens and presentation them (presentation) to activate specific immune response.
3. Secretion of the mediators of immune system by macrophage (monokines):

   - regulatory monokines (IL1)
   - monokines – effectors (participate in the process of intracellular killing).

The human immune system. Immunodiagnostic tests

Theme № 11

Composition (the anatomy) of human immune system: general scheme



Composition of immune system: immunocompetent organs

- **Central:**

☞Thymus

☞Bone marrow

Function: **formation, antigen-independent differentiation and proliferation of immunocompetent cells**

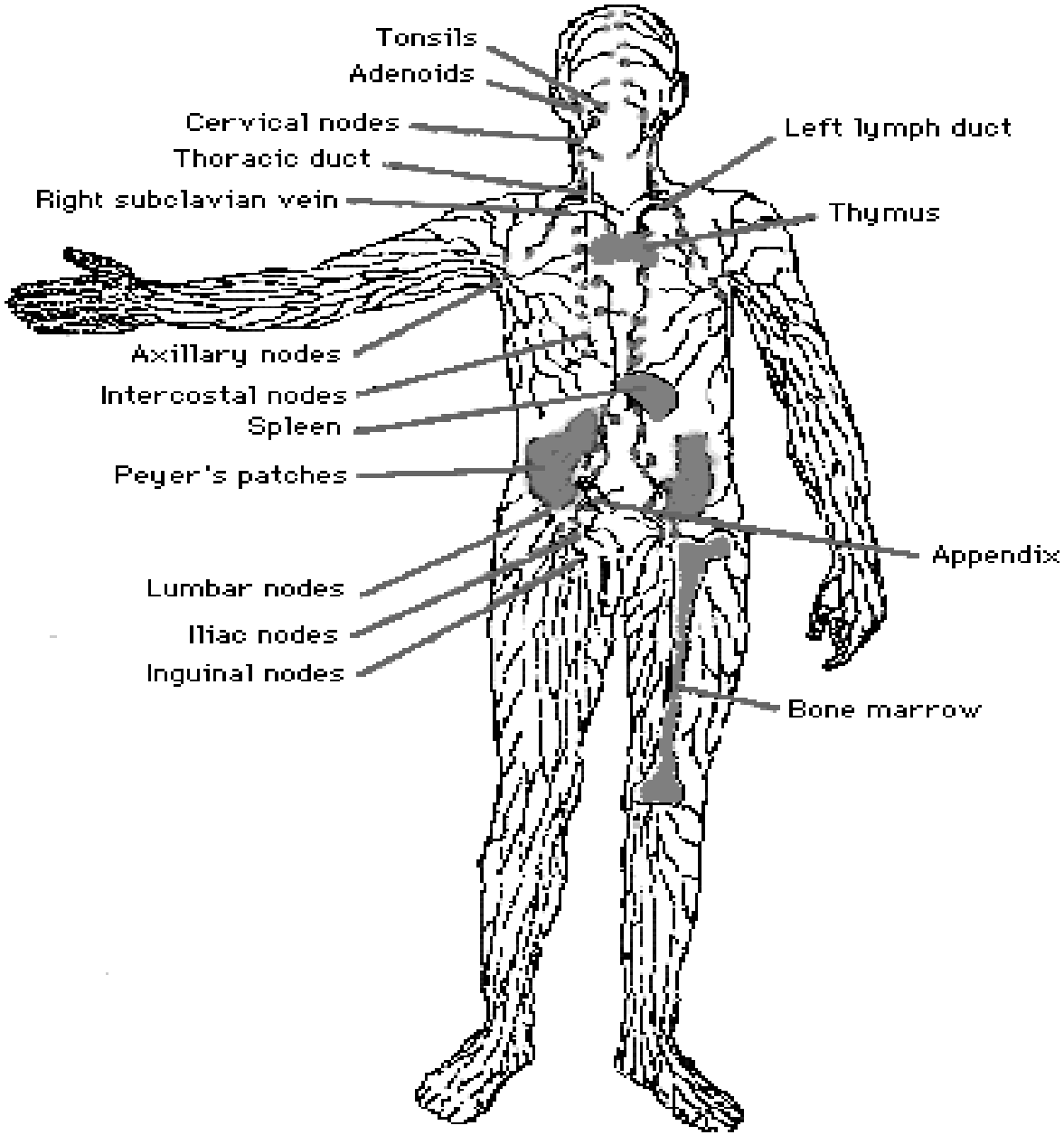
•Peripheral:

INCAPSULATED ORGANS

- Lymph nodes
- Spleen

MUCOSAL ASSOCIATED LYMPHOID TISSUE (MALT)

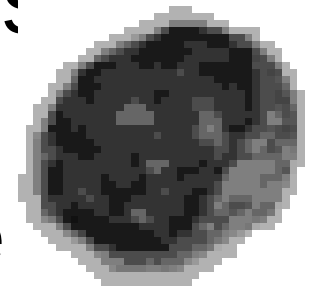
- Diffusive:
  a) aggregations of lymphocytes, phagocytes and plasma cells in lungs
  b) bronchus associated lymphoid tissue (BALT)
  c) gut associated lymphoid tissue (GALT)
  d) lymphoid tissue found in the urogenital tract
  e) naso-pharyngeal associated lymphoid tissue (NALT)
- Organized lymphoid tissue having follicular structure
  a) Waldeyer-Pirogov's ring – tonsils and adenoids
  b) Peyer's patches of intestine - large aggregates of lymphoid tissue found in the small intestine
  c) appendix


Tonsils
Adenoids
Cervical nodes
Thoracic duct
Right subclavian vein
Left lymph duct
Thymus
Axillary nodes
Intercostal nodes
Spleen
Peyer's patches
Appendix
Lumbar nodes
Iliac nodes
Inguinal nodes
Bone marrow

Peripheral :

- **Function: antigen-dependent (antigen-stimulated) differentiation and proliferation of immunocompetent cells**

Immunocompetent T- and B-cells: main characteristics



- **Lymphocytes** are produced within bone marrow.
- If they achieve immune-competence within the bone marrow, they are known as **B cells.**
- If they achieve immune-competence in the thymus they are known as **T cells**.
- Mature lymphocytes all have a similar appearance. They are small cells with a deeply basophilic nucleus and scanty cytoplasm.

Composition of immune system: immunocompetent cells and their functions

- **T-lymphocytes (cells)** – immune response and in particular – cell-mediated immune response
- **B-lymphocytes (cells)** – humoral immune response
- **NK-cells ("null" lymphocytes)** – antibody mediated cytotoxicity (AMCT)
- **A-cells** (macrophages) – presentation of alien antigen
- Dendritic cells - presentation of alien antigen

Immunocompetent T- and B-cells: main functions

- **B cells** respond to antigen and give rise to plasma cells which secrete immunoglobulins (antibodies):

  humoral immunity.

- **T cells** also respond to antigens:

1. T- cells CD4+ (helpers) secrete lymphokines which act on other cells involved in the immune response.
2. T-cells CD8+ (cytotoxic lymphocytes -CTL) cause lysis of infected cells- extracellular cytotoxicity.

T- helpers: main characteristics of subpopulations and their functions

Th_1 – activate cytotoxic lymphocytes and macrophages (induce cell mediated immune response and inflammation)

Th_2 – activate B-lymphocytes (induce humoral immunity)

Expression of the surface markers on T- lymphocytes

***CD* – surface molecules called clusters of differentiation**

- **CD3 –** found on the surface of all T-lymphocytes
- **CD4 –** T-helpers (Th_1 и Th_2)
- **CD8 –** T-killers/suppressors

***TcR* - antigen-binding receptor of T-cells**

Expression of the surface markers on B-cells

- **CD19 and CD21– clusters of differentiation of B-lymphocytes**
- **Antigen-binding receptor of immature B-cells - IgM (immunoglobulin M)**
- **Antigen-binding receptor of mature B-cells - IgD**

Macrophages

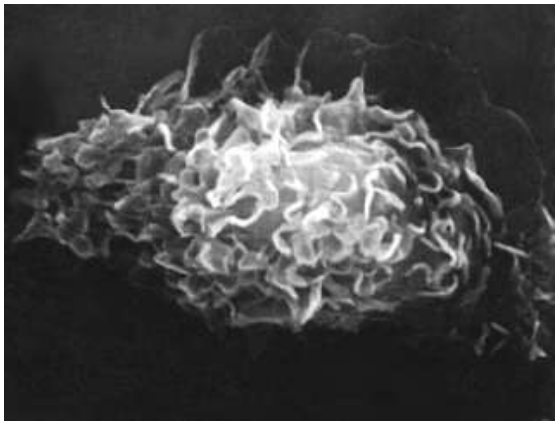
- **Tissue macrophages:**

1) Thymus
2) Liver - Kupfer cells
3) Spleen
4) Lymph nodes
5) Kidney - mesangial cells
6) Bone tissue – osteoclasts
7) Connective tissue
8) Mucosal associated lymphoid tissue
9) Brain – microglial cells

- **Macrophage-like cells**

1) Dendritic cells including Langengar's cells
2) Monocytes - circulate in the peripheral blood

Macrophages: functions



These cells are derived from the bone marrow and have a variety of functions in the immune response:

- phagocytosis
- antigen processing
- antigen presentation
- secretion of cytokines

MOLECULES OF THE IMMUNE SYSTEM

Receptor - ligand molecules localised at the surface of the immune cells:

Molecules belonging to the immunoglobulin family:

- **Ig (IgM and IgD –receptors of B-cells)**
- **TcR (receptor of T-cells)**
- **MHC (HLA) – major hystocompatability complex**
- **CD molecules (CD2,3,4,8, etc)**
- **adhesion molecules of immune cells (selectins, integrins, etc)**

MOLECULES OF THE IMMUNE SYSTEM

Circulating molecules – cytokines (the family of biologically active peptides)

- **interleukins (IL) 1-21**
- **colony stimulating factor (CSF)**
- **tumor necrosis factor (TNF)**
- **chemokins**
- **interferons**
  - **All these peptides possess hormone like effect and provide co-interaction between the cells of immune system, between immune system and blood system as well as nervous and endocrine systems.**

Immunodiagnostic tests

Immunodiagnostic tests (classification)

1. Serological reactions

   reactions of antigens (Ag) with antibodies (Ig) in vitro

2. Cell - mediated immune reactions

   reactions which involve immunocompetent cells

3. Hypersensitivity tests (allergy tests)

   revealing of hypersensitivity

Serological reactions (tests)

The purposes

- **to identify antigen**
  - **in specimen (express diagnostics)**
  - **in pure culture of microorganism**
    - **serological identification (to determine the species)**
    - **serotyping (to determine serological variant**)
- **to reveal antibodies (Ig)**
  - **the fact of their presence (qualitative) in the serum**
  - **quantitative test (show increasing of the titer of – method of antibodies («pair sera»)**

Serological reactions (tests)

Classification

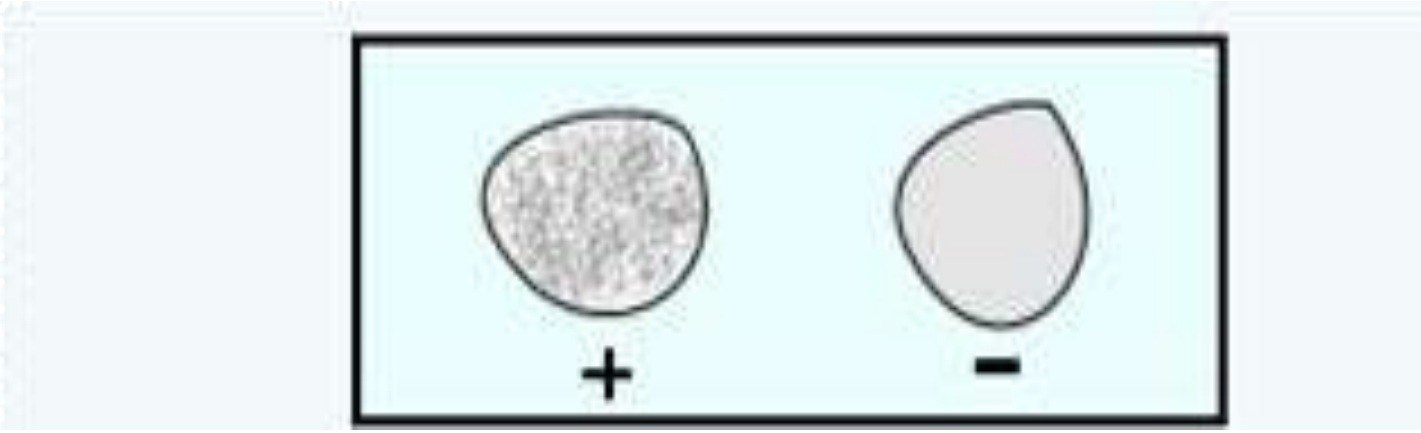
1. Simple (involve two components: Ag + Ig)
   - **RA – reaction of agglutination (with insoluble corpuscular antigen)**
   - **RP – reaction of precipitation (with soluble antigen)**
2. Complex – complement fixation reaction
   (three – component reaction: Ag + Ig + C)
3. Reactions occurring with use of labels.

Reactions of agglutination

involve insoluble corpuscular antigen

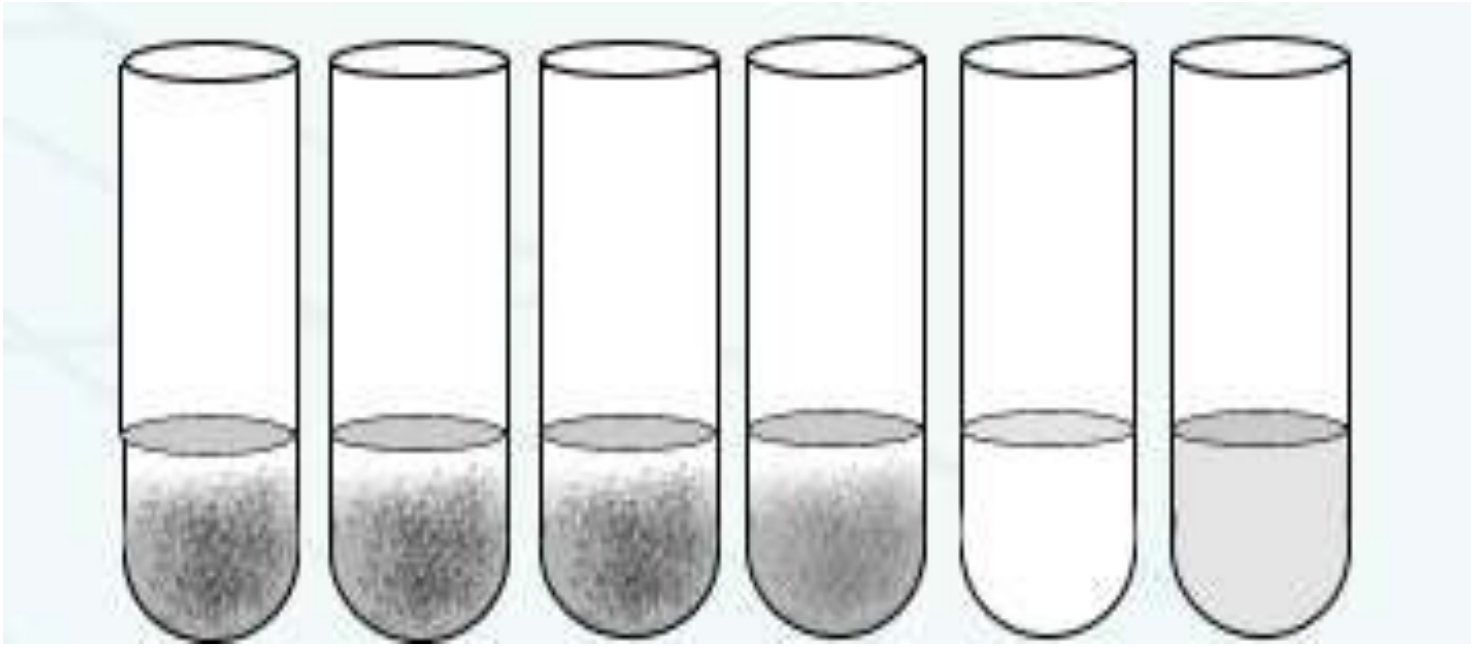
- ✓ **Direct**
  1. **Performed on the glass slide**
  2. **Performed in the test tubes with serial dilutions of antibodies – *tube agglutination test***

Agglutination on a glass slide



Positive reaction Control test (negative reaction)

Tube agglutination test



Positive result

Serum control test

Antigen control test

Reactions of agglutination

- **Indirect or passive**

(use passive carriers of antigen to make the reaction more visible):

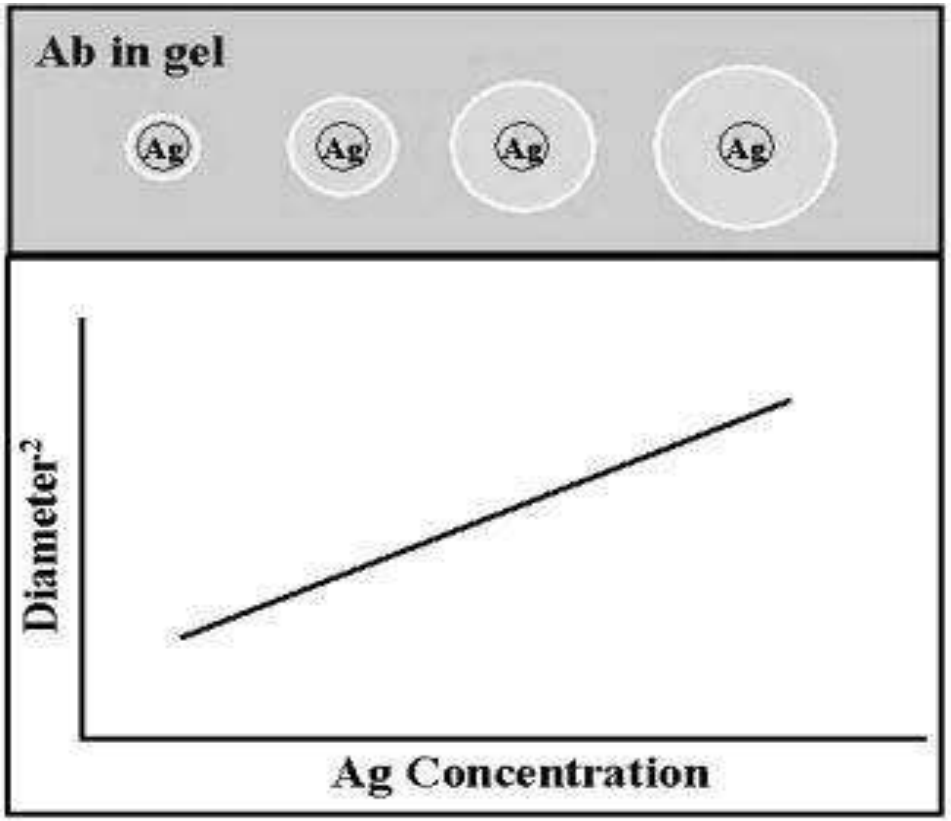
1. **Latex-agglutination (latex particle agglutination test) – antigen absorbed to latex beans**
2. **Co- agglutination - antigen absorbed to the cells of Staphylococci**
3. **Indirect (passive) hemagglutination reaction – antigen absorbed to erythrocytes**

Reactions of precipitation

Involve soluble antigen

- In test tubes
- In gel (immunodiffusion) – the reaction between antigens and antibodies that resulted in a complex formation which is visible as a precipitate (lines of precipitation) in agarose medium
    1. **Single (Mancini technique): one reactant fixed in the medium and the other is allowed to diffuse**
    2. **Double (Ouchterlony technique): both reactant diffuse toward each other**
- Reaction of the toxin neutralization by antitoxic serum (RN) performed in the test tubes
- Other variants
    1. **Immunoelectrophoresis**
    2. **Immunoblotting**

Single immunodiffusion (Mancini technique)

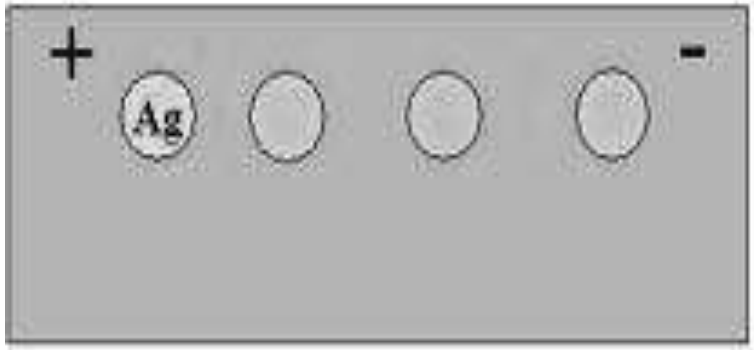


1. Ab is incorporated into the agar gel.
2. Different dilutions of the antigen are placed in holes punched into the agar.
3. The Ag diffuses into the gel, it reacts with the Ab and a ring of precipitation is formed.

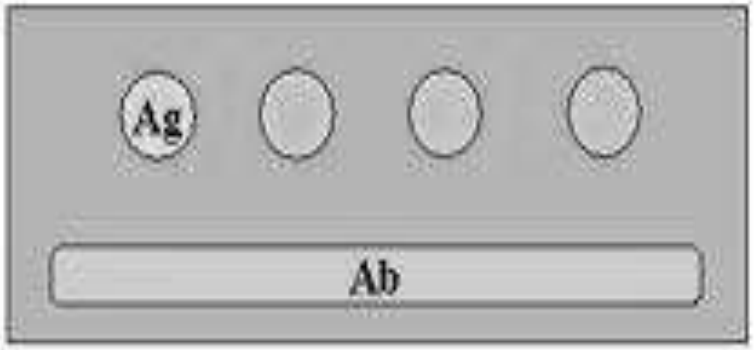
Immunoelectrophoresis

- The test carried out on glass slides covered with agarose provides separation of complex protein antigens (from serum, urine, others sources) in an electric field and their diffusion towards the antibodies in semisolid medium with formation of lines of precipitation.

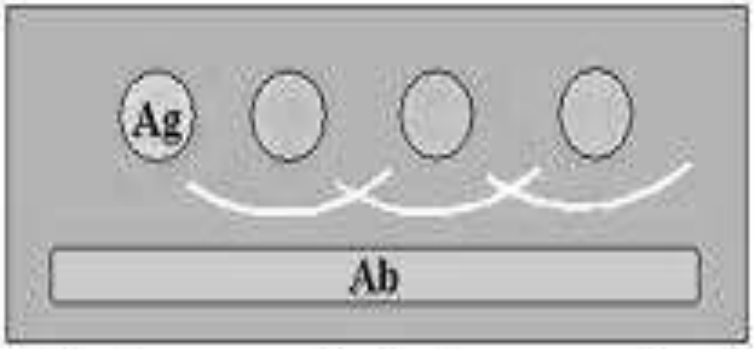
Immunoelectrophoresis



Mixture of Ags is electrophoresed to get separated Ags according to their charge



A trough is cut in the gel and Abs are added



The Abs diffuse into the agar, precipitin lines are produced in the zone where an antigen/antibody reaction occurs

Complex serological reactions

Include three components : Ag+Ig+C

1. Visible
    1. Immobilisation
    2. Immune sticking
    3. Lysis (including hemolysis)
2. Invisible
    1. Complement fixation reaction (CF)

REACTIONS OCCURRING WITH USE OF LABELS

- IFR immunofluorescence reaction
- ELISA enzyme-linked immunosorbent assay
- RIA radioimmunoassay
- IEM immunoelectronic microscopy

Cell - mediated immunity. Antigens.

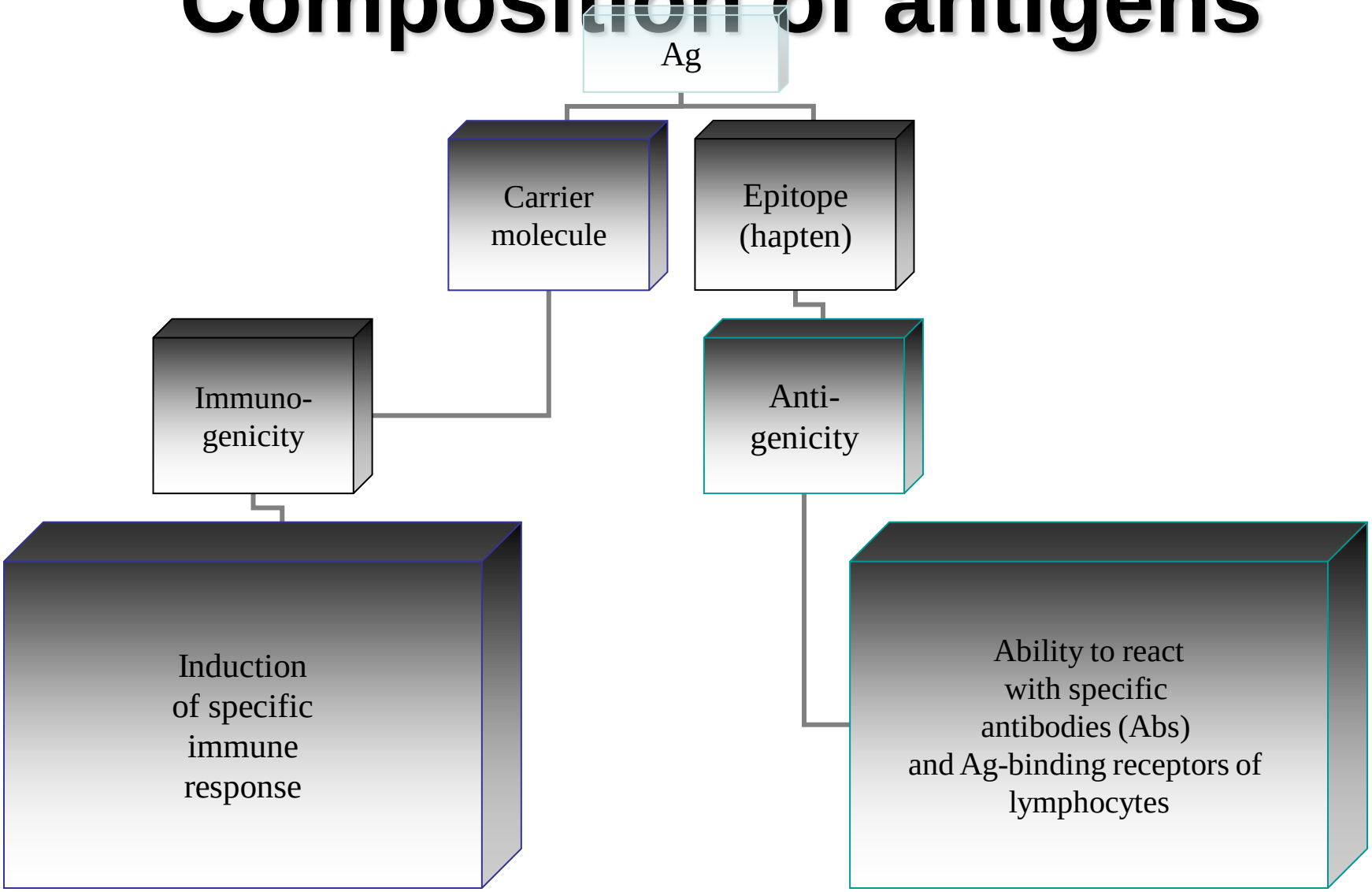
Theme № 12

ANTIGENS

Antigens (Ags): definition of the term

- Foreign molecules (substances), which are distinguished by the immune system as alien in the context of «own/alien» (“self-non-self”).

Composition of antigens



Epitope

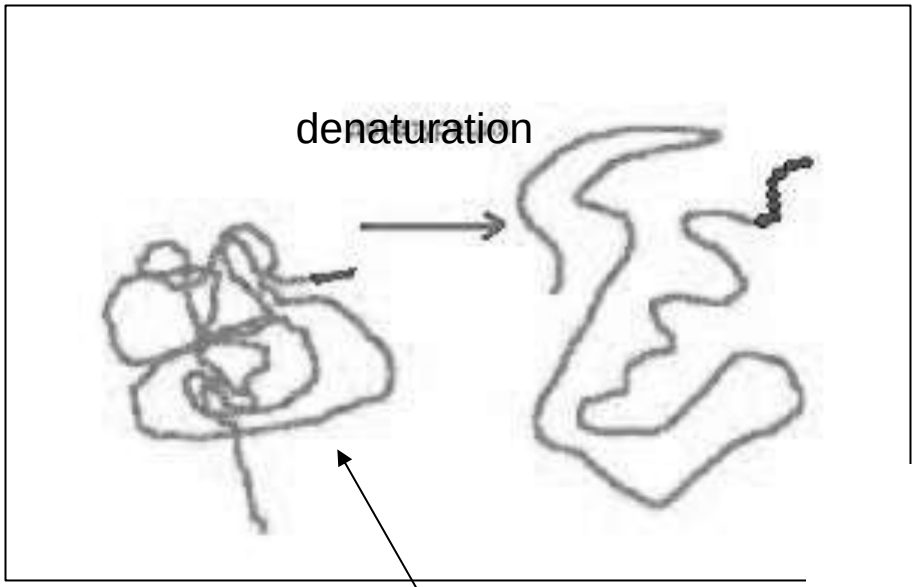
Definition of the term

- **Part of the antigen, low molecular weight molecules : peptides, carbohydrates or lipids having specific structure and configuration (specific reactive sites) which localized at the surface or within the molecule of antigen**

Synonyms:

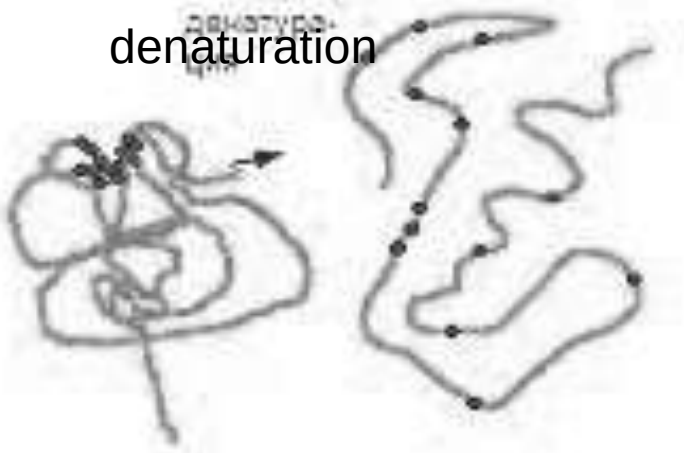
- **«antigenic determinant»**
- **«determinant group of antigen»**

Composition of antigens



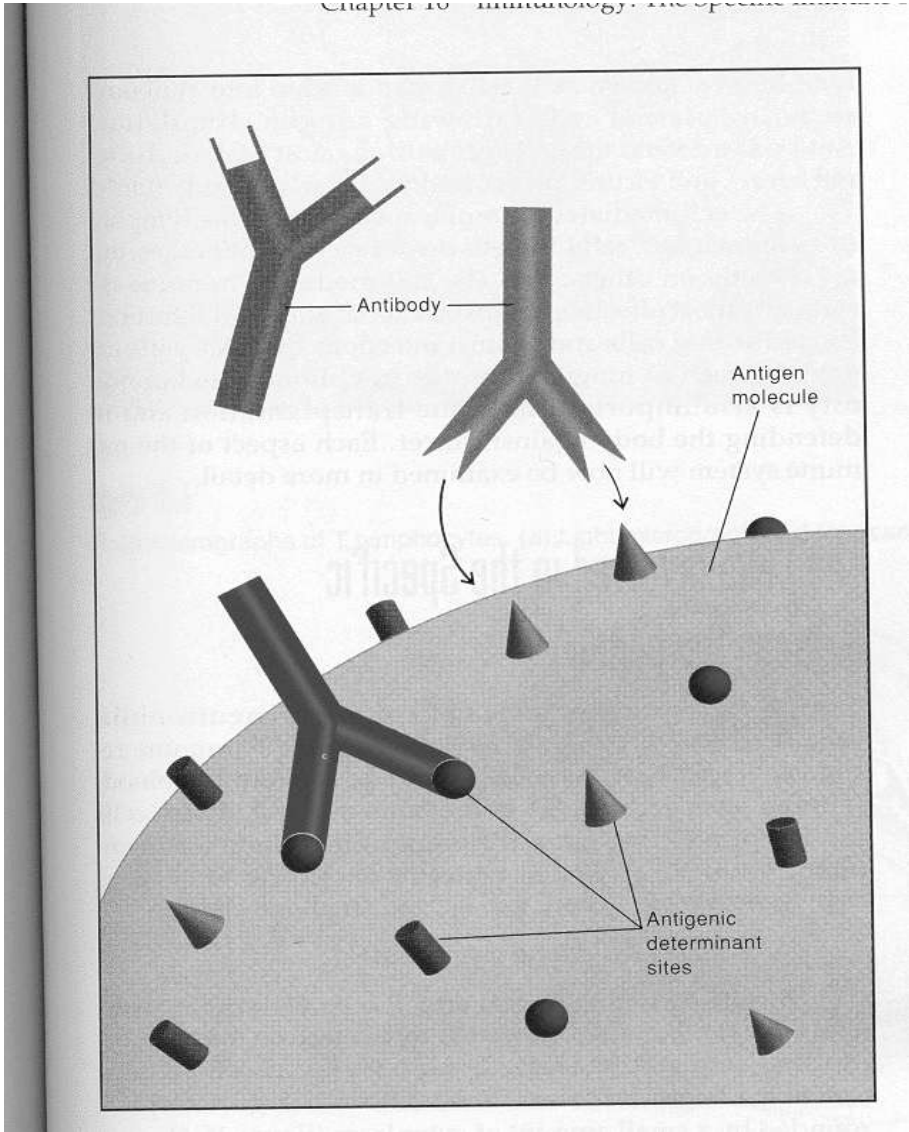
confromational

linear



EPITOPES

- **Antigen binding site of Ab has a configuration complementary to the shape of epitope**



Factors which determine the immunogenicity of antigen

- physico-chemical properties of antigen
- the dynamics of inflow of antigen into macro-organism and the dynamics of its catabolism in the macro-organism
- physiological state of macro-organism at the moment of the contact with the antigen
- introduction of antigens into macro-organism in the complex with adjuvants

Adjuvants

- **The substances (mineral oil, mixture of waxes, etc) which possess the ability to enhance the immune response to antigens nonspecifically: involving such mechanisms as creating of «depot of antigens». They enhance immunogenicity when mixed with antigen.**

AUTOANTIGENS

- **OWN ANTIGENS (SELF ANTIGENS) OF HUMAN ORGANISM.**

Normally they don't induce specific immune response (the sate called specific nonresponsiveness to self-antigens) because of two main possible reasons:

1. lack of immunocompetent cells possessing antigen-recognizing receptors to self-antigens (**immunological tolerance**) – developed during fetal life;
2. some tissue antigens are normally isolated from the immune system by anatomical barriers («OVER BARRIER ANTIGENS»).

Antigens of the major histocompatibility complex (MHC): main characteristics

MHC or HLA (in humans MHC called human leukocyte antigen - HLA)

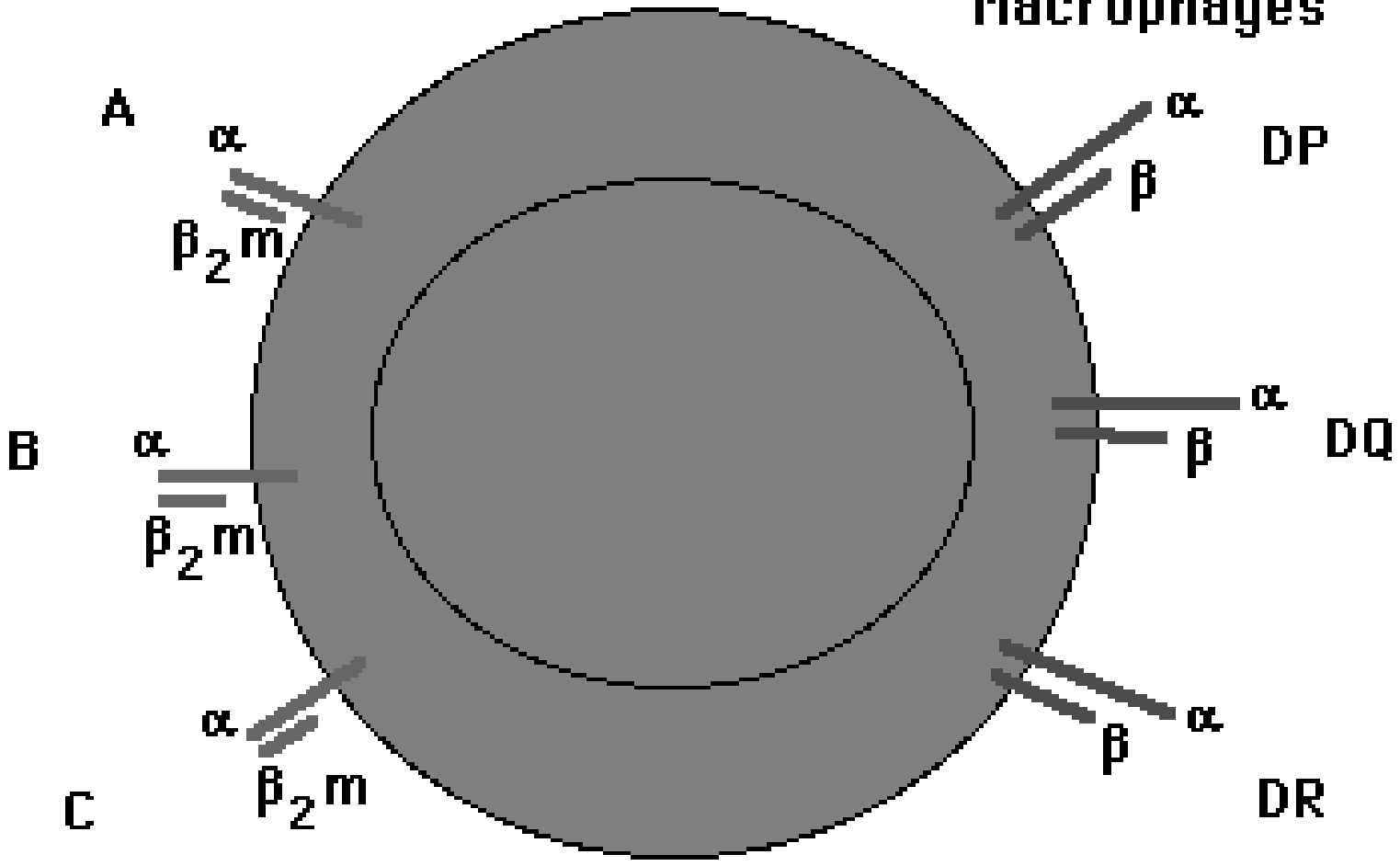
- glycoproteins tightly attached to the surface of mammalian cell membrane which play a key role in the realisation of the mechanism of recognition of antigen by immune system
- class I MHC molecules are expressed constitutively on almost all nucleated cells of the body
- expression of class II MHC molecules is restricted mainly to certain cells of the immune system - B cells, macrophages and dendritic cells

MHC: antigen presentation

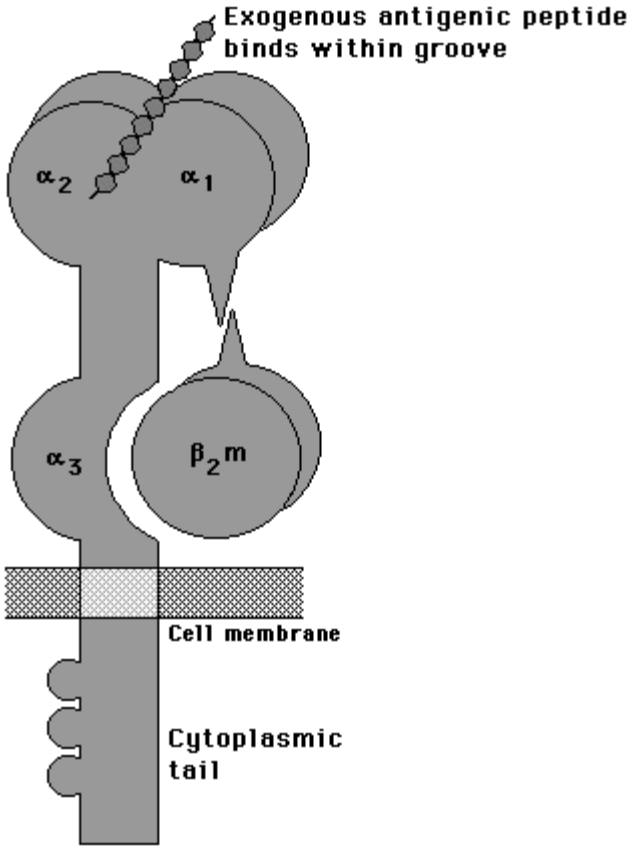
- antigen could be recognized by the immune system just in a complex with **MHC** (when antigen bound to MHC)
- T cell recognition of antigen involves direct cell-to-cell contact between the antigen-specific TcR on the T lymphocyte and an MHC/peptide complex at the surface of antigen presenting cell
- in general, class I MHC molecules present antigen to CD8+ T cells, and class II MHC molecules present antigen to CD4+ T cells
- antigens bound for presentation in association with MHC class I molecules are derived from the cell's cytosol (for instance, viral antigens or pathogens – intracellular parasites)

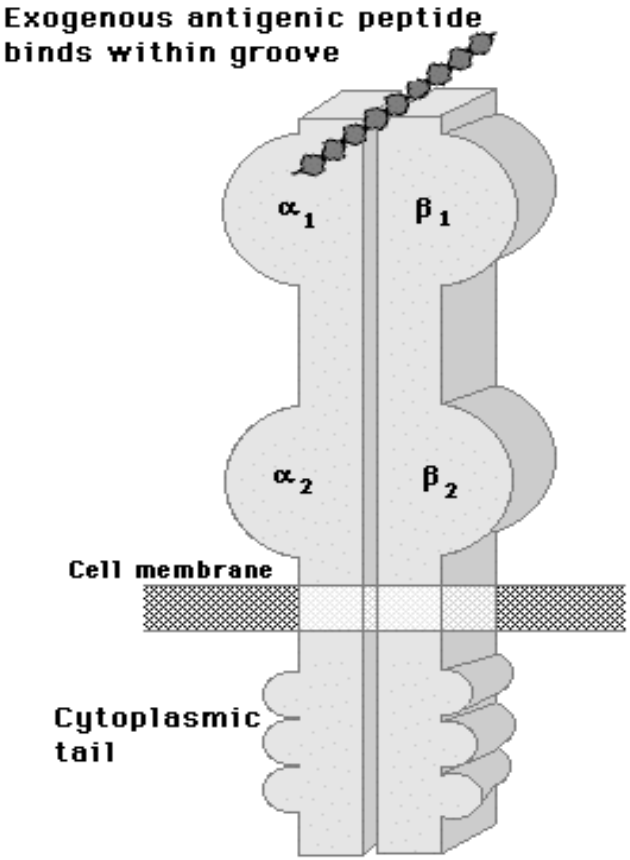
MHC Class I:
All nucleated cells

MHC Class II:
B cells
APC's
Macrophages



MHC class I and class II





Bacterial antigens: classification according to their specificity

- **Group-specific** (common for several different species).
- **Species - specific** (common for one species).
- **Type-specific** (common for one serological variant).

Bacterial antigens:

classification according to their nature (origin)

- Cell debris (parts of bacterial cell, cellular components).
- Products of vital activity of bacterial cell.

Antigens – organelles of bacterial cell (cell debris)

- CELL WALL
  - **O-Ag**
    - **Gram-positive bacteria**→ teichoic acids
    - **Gram-negative bacteria**→ **LPS**
- CAPSULE
  - **K-Ag**
  - **Vi-Ag** (capsule antigens in Salmonella typhi)
- FLAGELLAS
  - **H-Ag**
- OTHERS

Antigens – metabolic products of microbial cells (products of the vital activity)

- Protein toxins.
- Enzymes.
- Protective antigens (nontoxic proteins –components of the peptide capsule in anthrax rods, usually strongly immunogenic).

CELL – MEDIATED IMMUNITY

Cell – mediated immunity: main stages

1. Interaction of the target cell **(** it could be cell infected by virus or other microbe – intracellular parasite) carrying alien surface proteins that are expressed on the surface in combination with class I MHC.
2. Presentation of the processed antigen (small peptide fragment of Ag) associated with class I MHC molecules at the surface of the target cell to cytotoxic CD8+ T lymphocytes (CTL) – **the first signal of activation for CTL.**
3. Activation of CTL.
4. The signal sent by antigen-presenting cells (APC) involved into cellular immune response to activate Th1 cells:
   - APC (it could be macrophage, dendriric cell or B cell) carrying MHC II interacts with antigen.
   - The step of processing – degradation of the antigen inside of the APC cell takes place.
   - The next step is presentation of the processed antigen (small peptide fragment of Ag) associated with class II MHC molecules at the surface of the APC to helper CD4+ T lymphocytes (Th1).
5. Activation of Th1.
6. Synthesis of cytokines (IL-2) by activated Th1.
7. **The second signal is distant signal**: activation of CTL by IL-2 sent by Th1.
8. Realisation of the effector part of cell – mediated immune response by activated CTL which leads to:
   - destruction of the cells carrying alien antigen by CTL (T killers) – clearance of infection
   - immune inflammation

Processing of the antigen

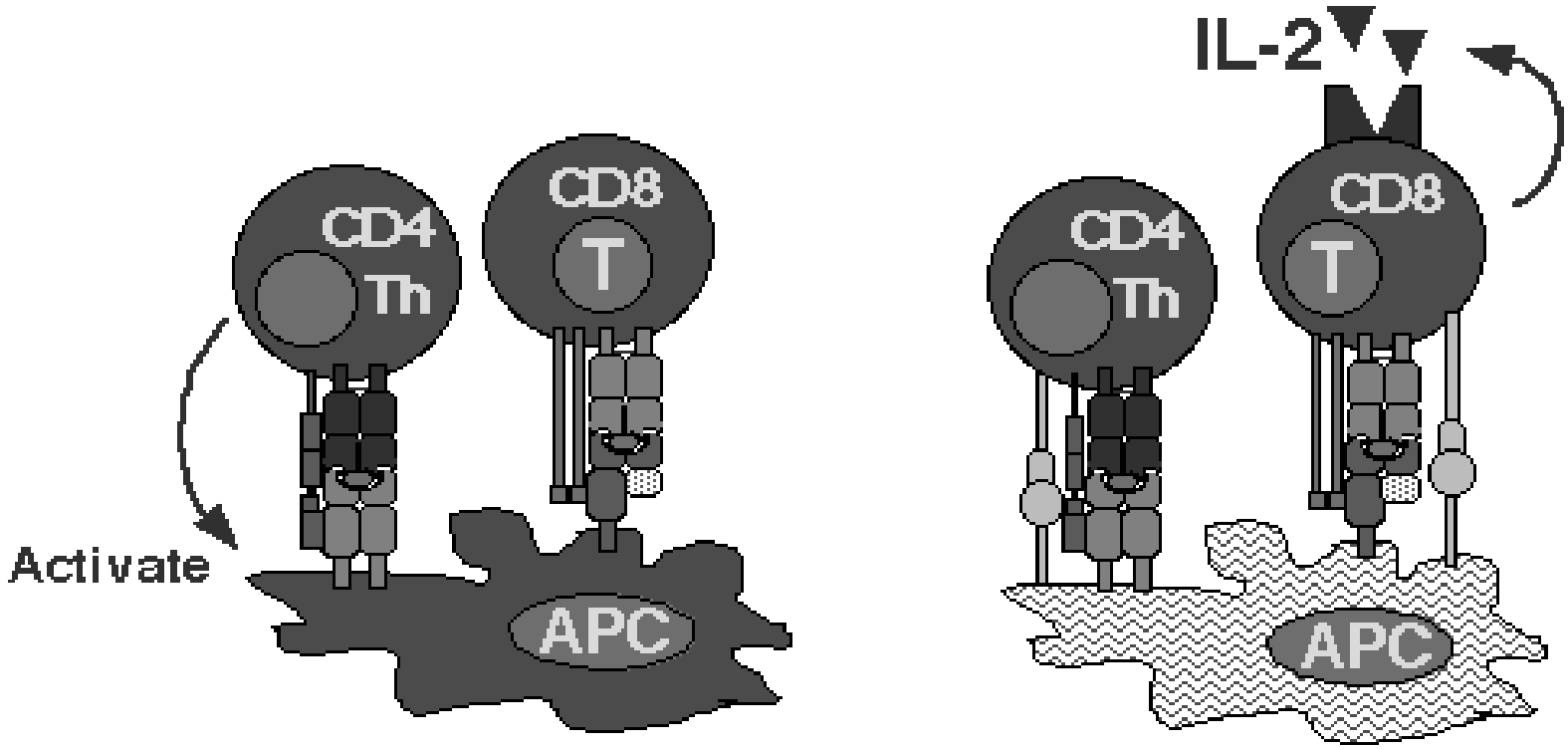
- Ingestion of exogenous antigen by APC.
- Partial degradation of the antigen.
- (that is the process of fragmentation of native antigen to isolate the epitope – usually short peptide fragment).
- Target cell (cell infected by virus or intracellular bacteria – parasites) uses epitopes (fragments) of endogenous antigens.
- Arrangement of the complex of epitope+ MHC (class I or II) which called «processed antigen» in the cytosole of the APC or target cell .
- Transport of MHC - antigen complex to the surface of APC or target cell and expression of «presented antigen».

Activation of T-lymphocytes

Activation signals obtained by T cells:

1. Activation of CD8+ cytotoxic T cells as a result of their tight contact with target cell involving complex MHC I + presented Ag on the surface of the target cell and TcR of CTL.
2. Interaction of the complex: MHC II + presented antigen on the surfece of APC with CD4+ Th1 cell through tight contact with TcR (signal is transmitted to the genome of Th1 by means of CD3 molecule).
3. Secretion of cytokine IL-2 by Th1.
4. Activation of CD8+ cytotoxic T cells by IL-2.

Indirect arming of CD8+ cytotoxic T cells by CD4+ helper cells



Signal 1 activated CD4+T cells activate APC to express costimulatory molecules

APC that express costimulatory molecules deliver signal 1 & 2 to CD8+ CTL that then make their own IL-2 for clonal expansion

Basic scheme of cell-mediated immune response

Antigen

⇓

presentation of Ag by target cell to CTL – contact signal for activation

⇓

presentation of Ag by APC to T-helper 1 cells

⇓

activation of Th1 cells

⇓

synthesis of IL-2 by activated Th1 cells – distant signal for CTL activation

⇓

activation of CTL (as a result of getting contact and distant signals)

⇓

delivery of lethal hit by CTL

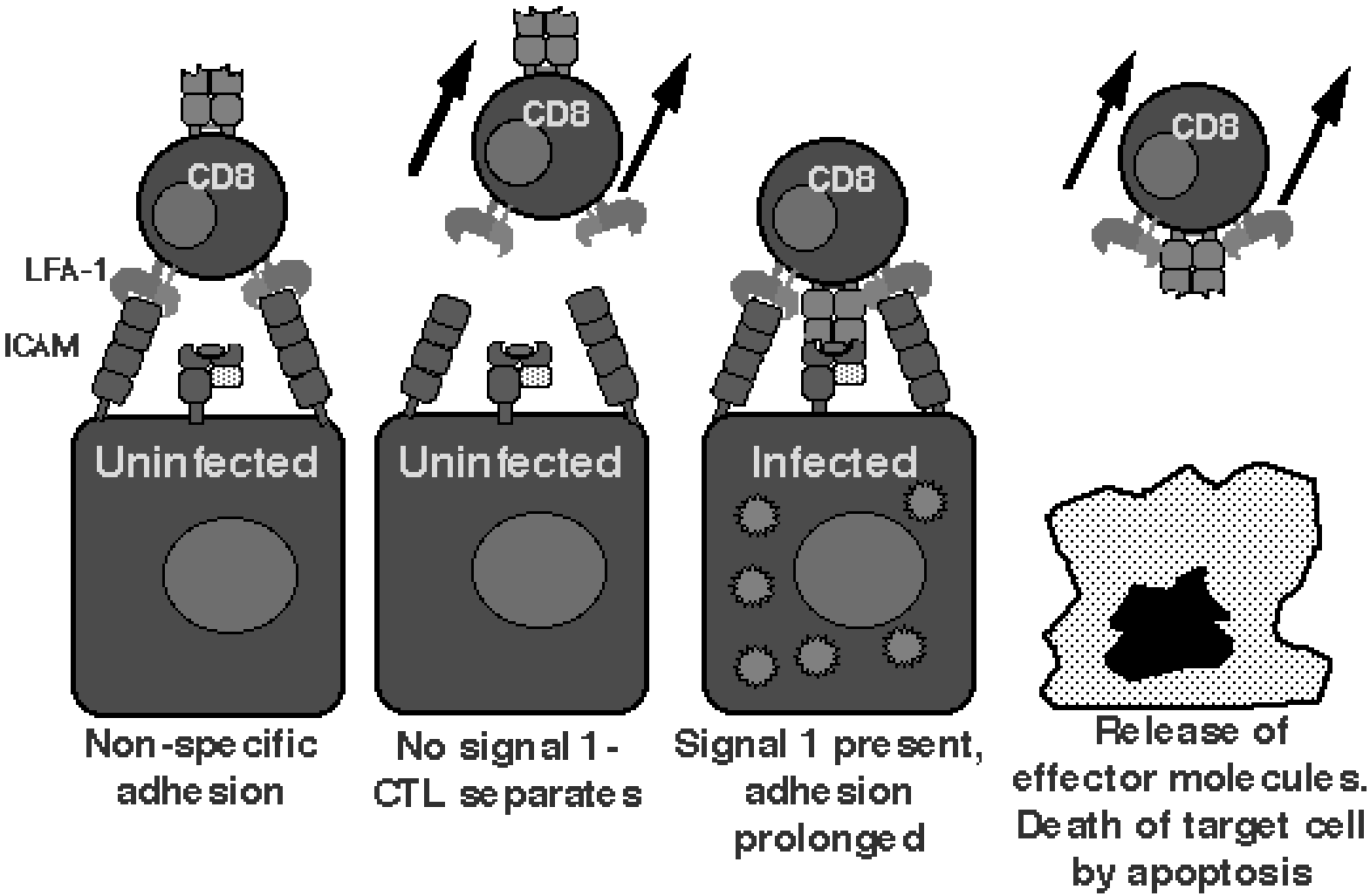
⇓

release of CTL from target cell

⇓

death of the target cell

CTL:target cell interactions are initiated by antigen non-specific molecules



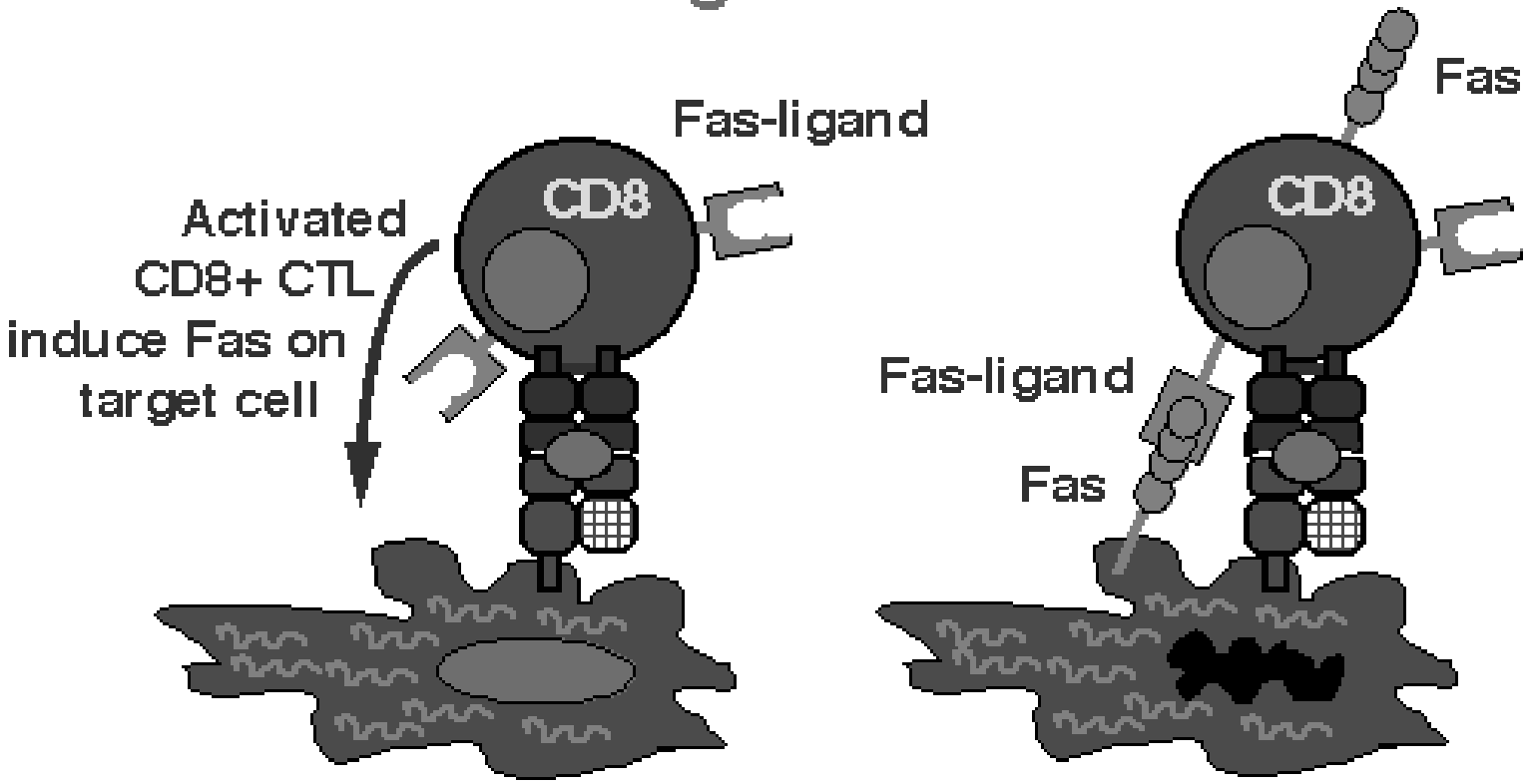
Mechanisms of killing by cytotoxic lymphocytes (T-killers)

1. Extracellular cytotoxicity which is realised through secretion of perforins (lethal hit) by CTL (perforins polymerise to form pores in target cell membrane) – necrotic cell death.
2. Activation of apoptosis (genetically encoded death) in the target cell – apoptotic cell death.

NECROTIC CELL DEATH

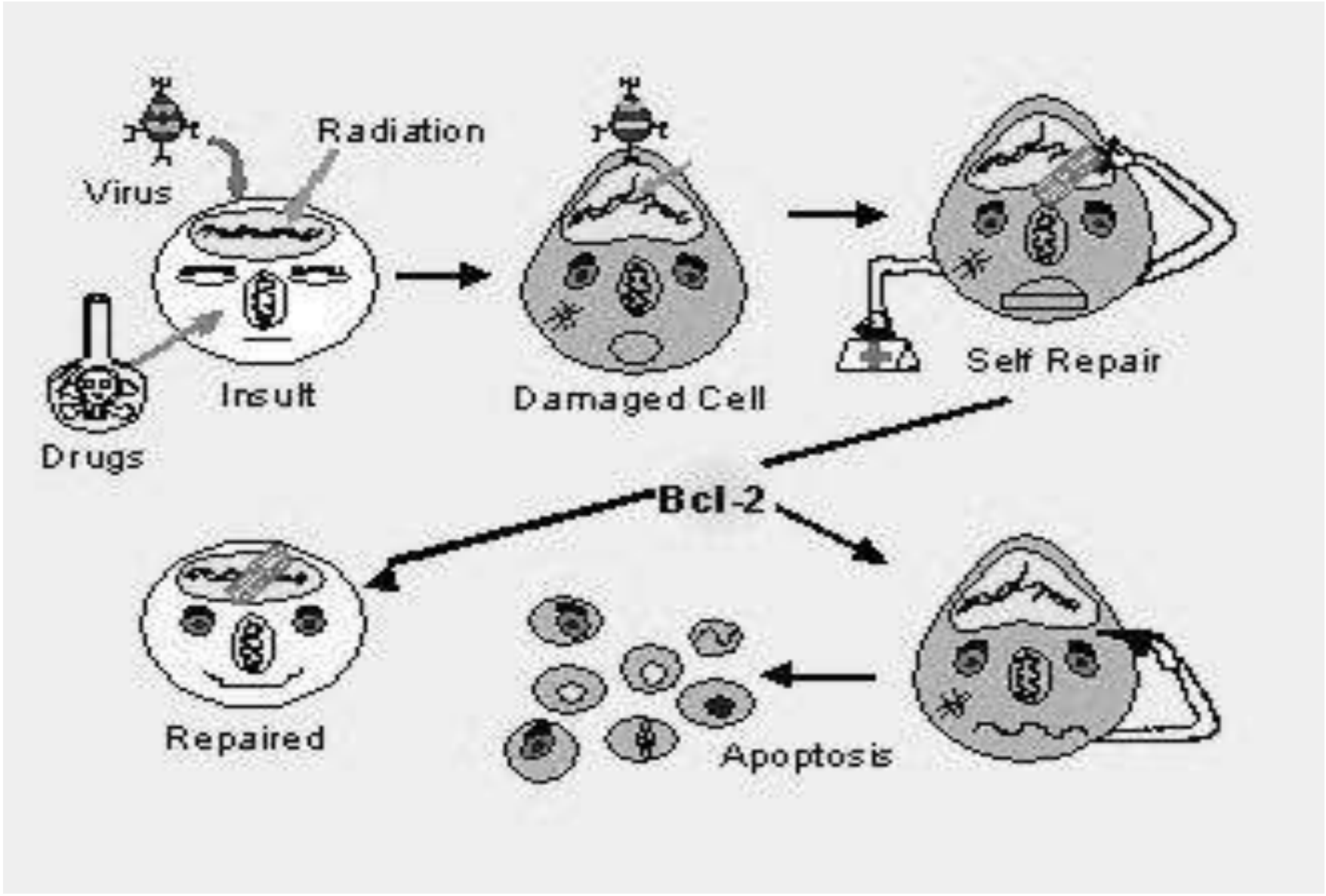
- Chemical or physical injury e.g.: oxygen deprivation in heart attack, antibody and complement damage.
- Dead cells are phagocytosed.
- Often triggers inflammation.

Cell killing by CTL membrane proteins: Fas-Fas ligand interaction



Virus-infected cell

Fas-Fas-ligand interaction induces apoptotic death in Fas-bearing target cell


Virus
Radiation
Drugs
Insult
Damaged Cell
Self Repair
Bcl-2
Repaired
Apoptosis

APOPTOTIC CELL DEATH

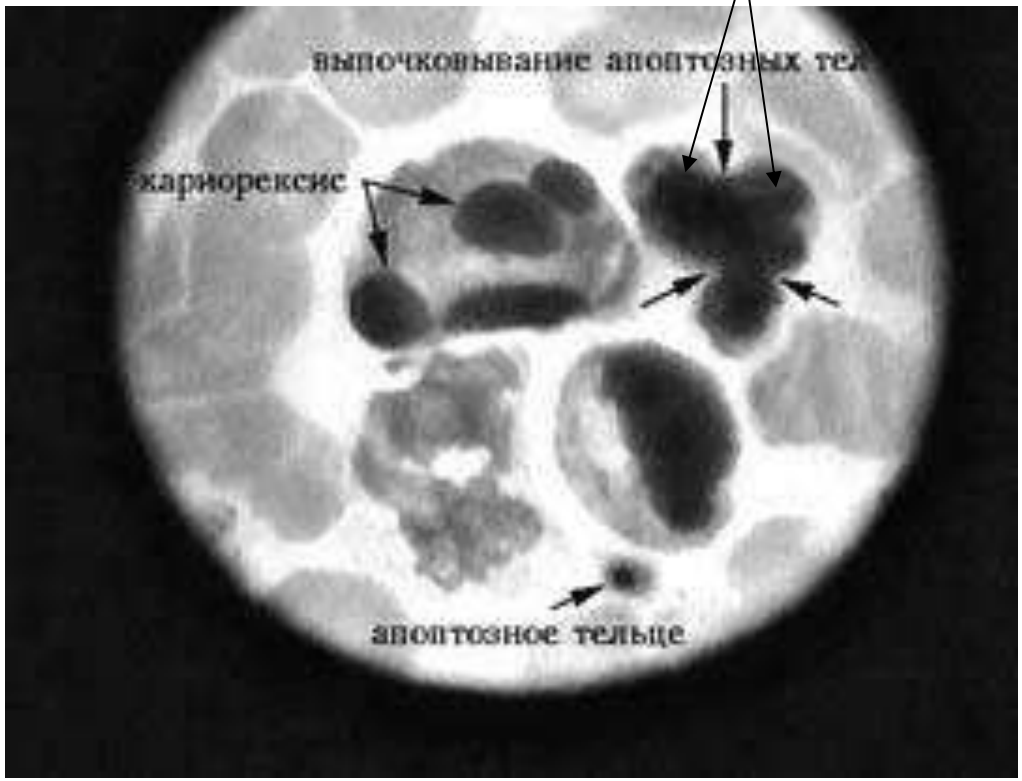
A normal cellular response – programmed cell death. Crucial in tissue remodelling during development and metamorphosis. DNA is cleaved and fragments of cells form apoptotic bodies.

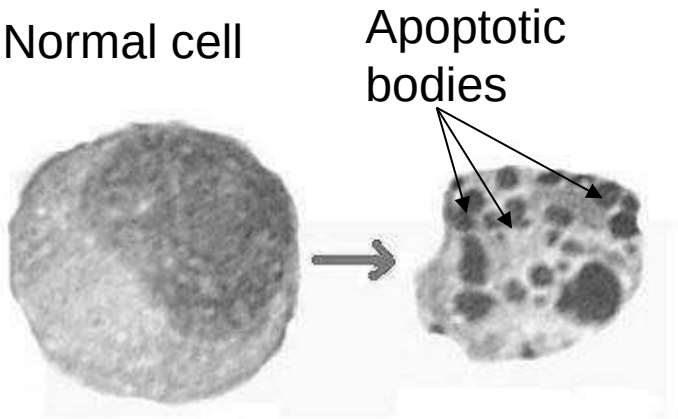
Apoptotic bodies contain “walled off” fragments of cells that are phagocytosed.

Not usually a trigger of inflammation.

Apoptotic cell death

Appearance of apoptotic bodies





REACTION OF AGGLUTINATION

REACTION OF AGGLUTINATION: BASIC TERMS

- **Antigen – *agglutinogen***
- **Antibody – *agglutinin***
- **Immune complex – *agglutinate***

Conditions of the proceeding of the reaction of agglutination

- **ANTIGEN –**
  should be at least tetravalent
- **IMMUNOGLOBULIN –**
  should be at least bivalent

Conditions of the proceeding of the reaction of agglutination

- **Reaction of agglutination (RA)** – is the process of binding of a particulate antigen (Ag) to specific antibody (Ig) in vitro leading to clumping of the antigen and forming **of the giant complex which is usually visible by unaided eye.**

Stages of the reaction of agglutination

- SPECIFIC (invisible) – forming of the lattice (clumps of antigen-antibody complexes – or carcass).
- NONSPECIFIC (visible) –the process of forming of the giant precipitates of the clumps of immune complexes visible by unaided eye.

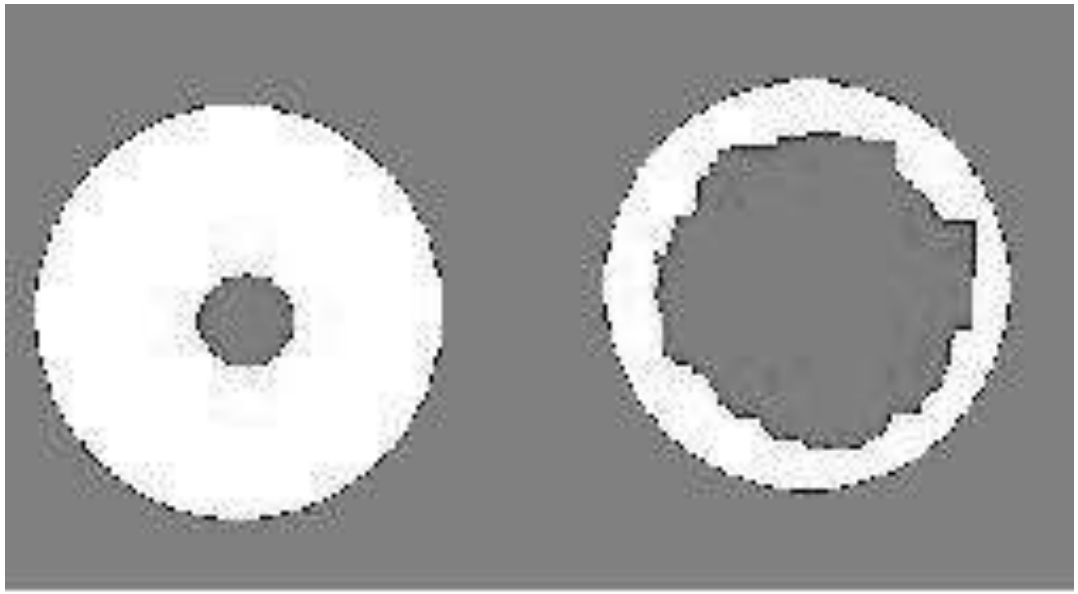
Visible displays of the RA

- **Finely divided agglutination** could be detected only with use of agglutinoscope or microscope – *reaction of micro-agglutination.*
- **Large flocculated** – for example, when agglutination of bacteria having flagella occurs, could be detected by unaided eye.

Passive hemagglutination reaction

- Red blood cells (erythrocytes) present a convenient surface onto which many types of Ags can be absorbed.
- Such coated cells will clump when mixed with Abs to these specific Ags – process called ***passive hemagglutination.***

Visible displays of passive hemagglutination reaction



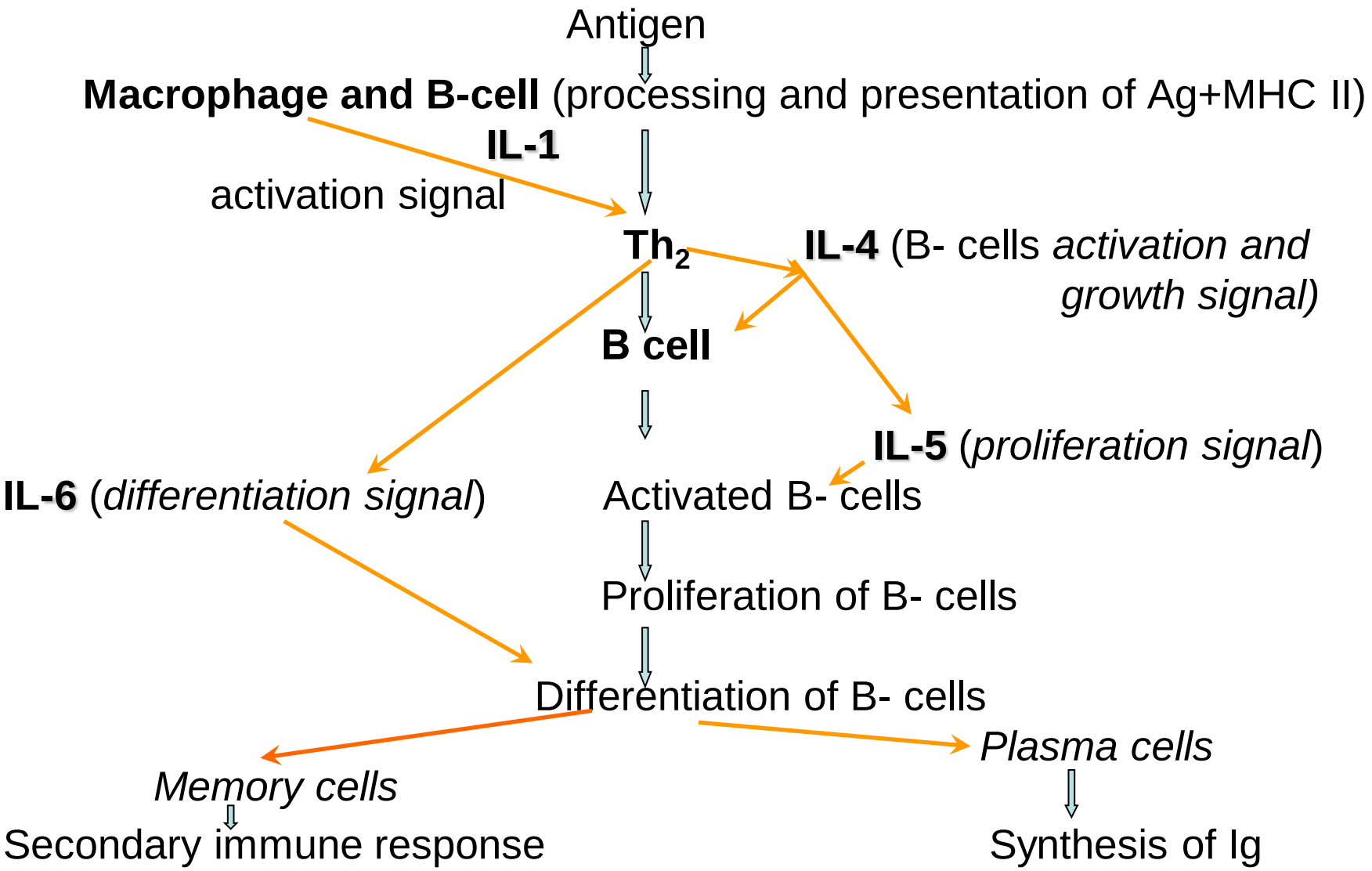
negative **positive**

HUMORAL IMMUNITY. IMMUNOGLOBULINS.

Theme № 13

HUMORAL IMMUNITY (the mechanisms of B-lymphocyte activation)

Activation of B-lymphocytes (B- cells)

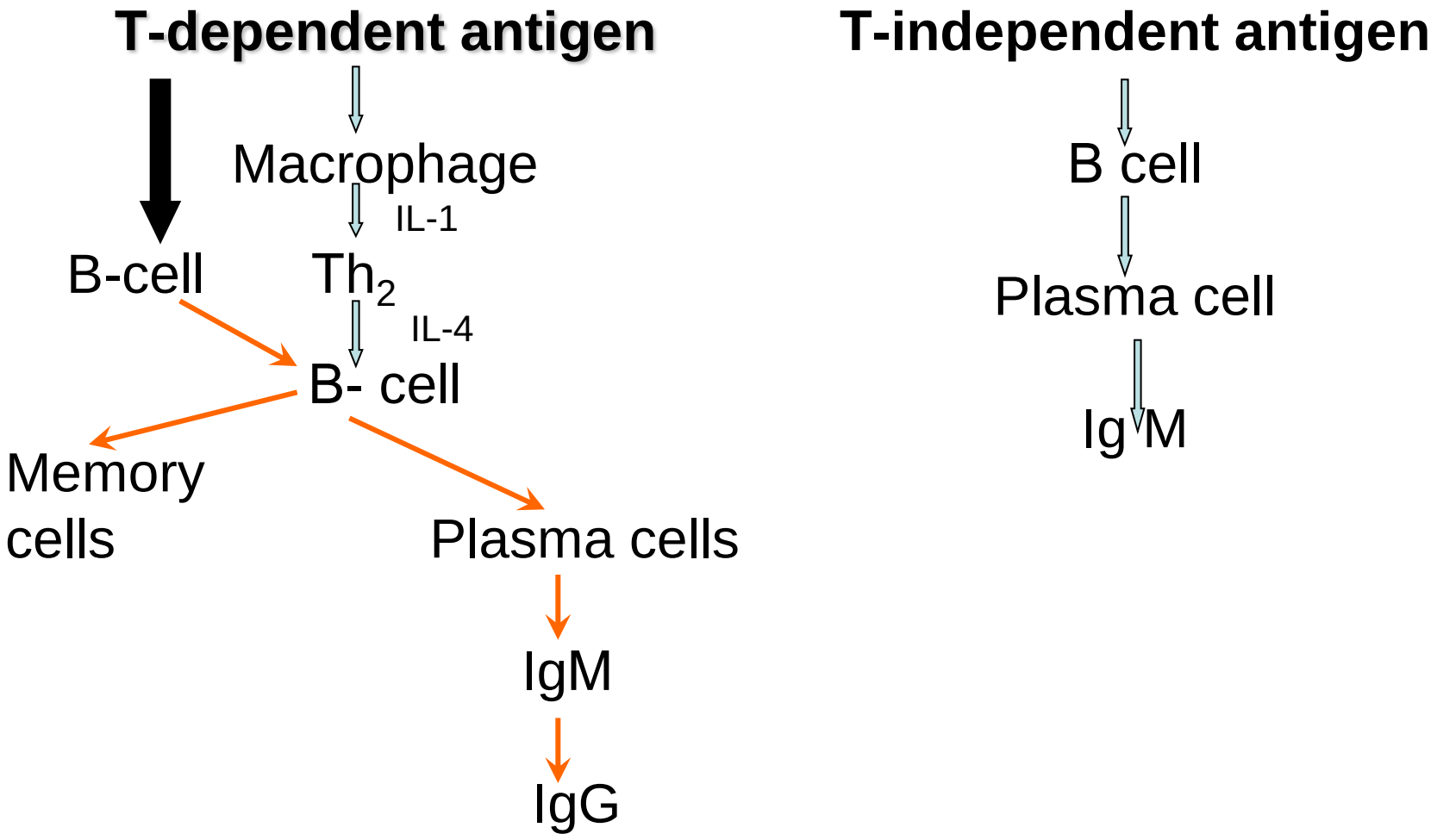


Activation of B-lymphocytes

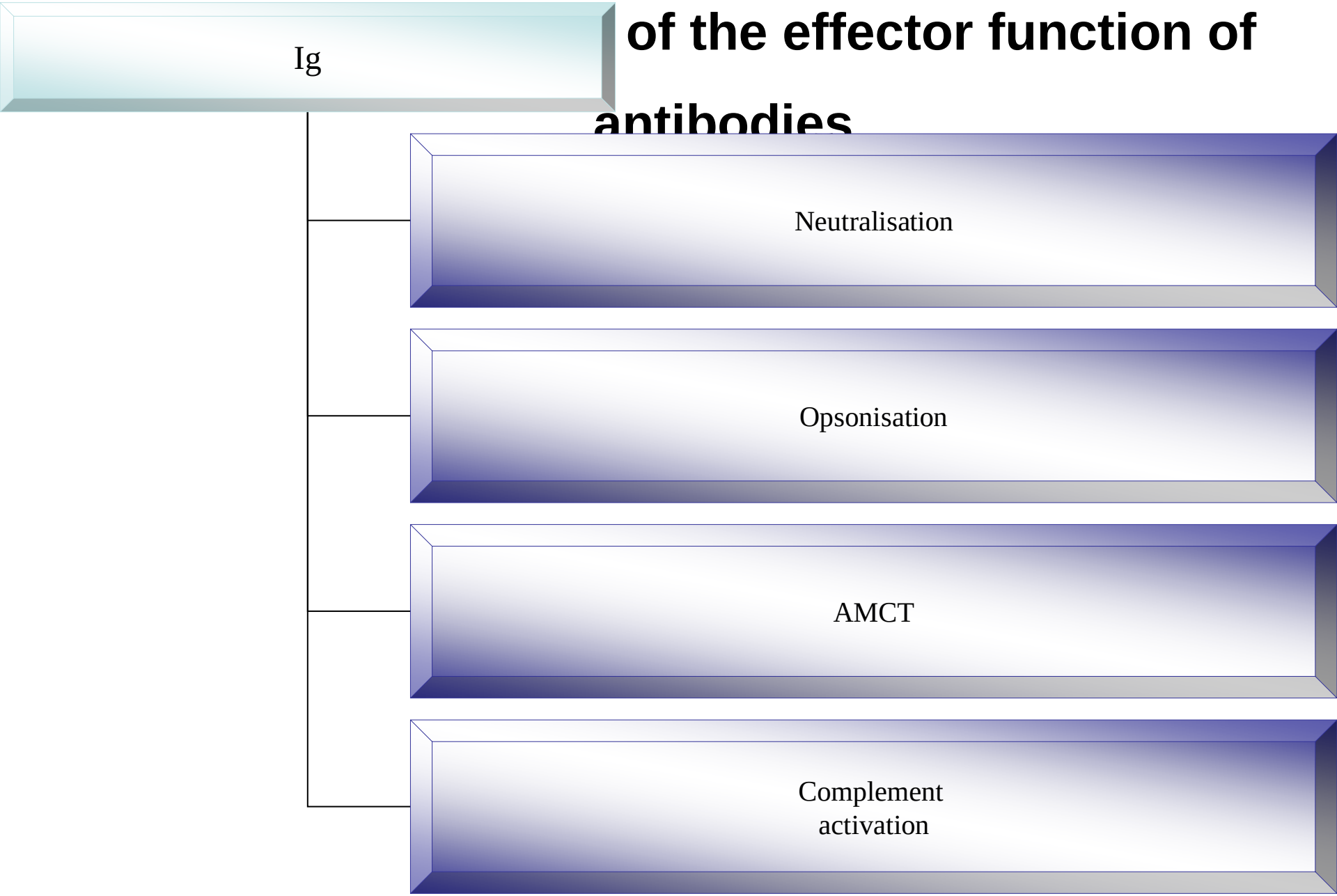
B cell is an antigen presenting cell itself

- B-cell receptor binds antigen.
- B-Cell acts as an APC: endocytoses, processes and presents that antigen to a Th cell activating it.
- Th-B-cell conjugate forms, which induces directional release of cytokines by the Th cell.
- Proliferation and differentiation of B-Cells occurs in response to the cytokines
- B-cells forming plasma cells that secrete antibodies for that antigen.

The general scheme of humoral immune response



of the effector function of antibodies

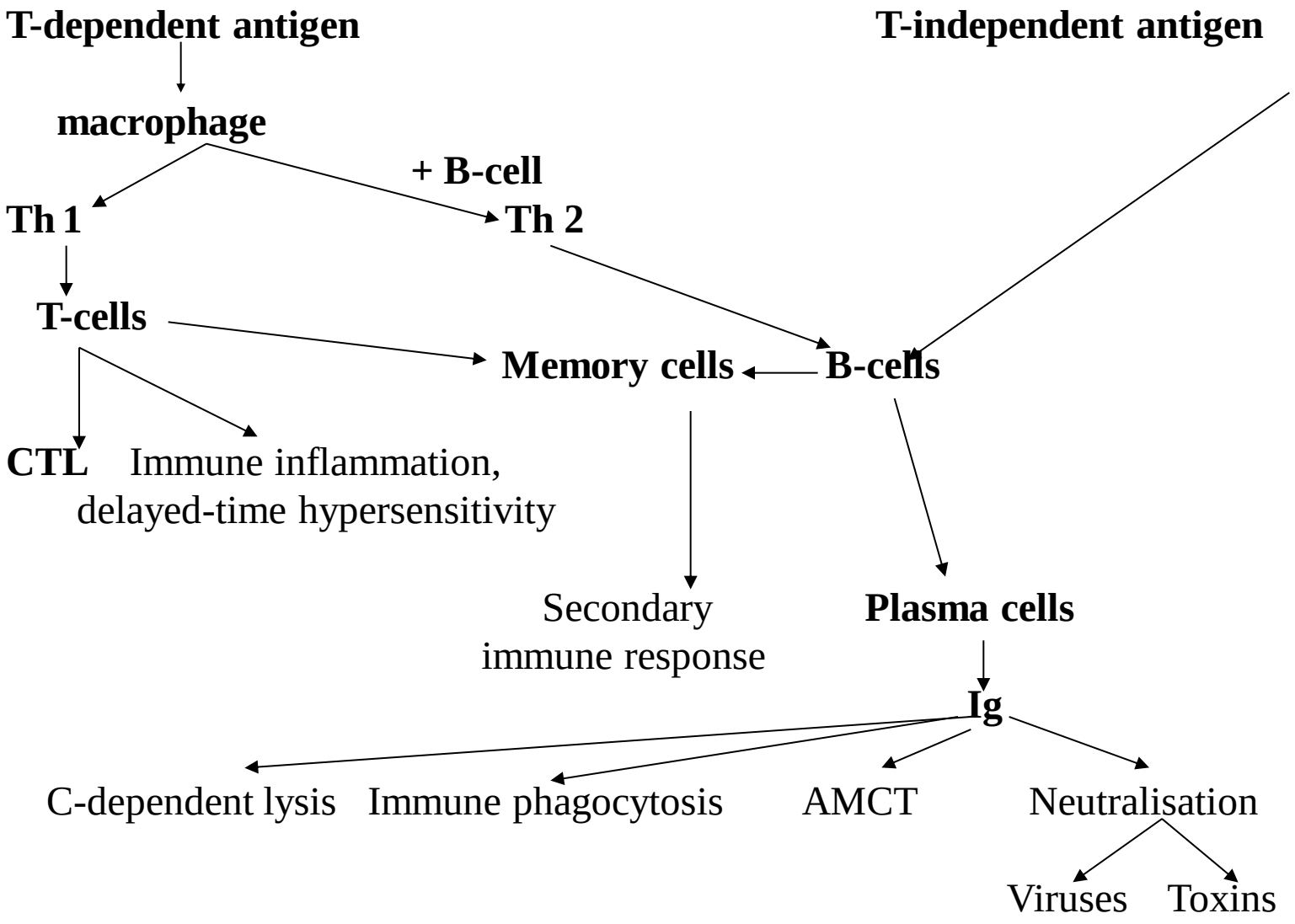


The mechanisms of the effector function of antibodies

Such effector functions include:

- ***Neutralisation*** **–** binding to viruses to neutralise their adsorption on the host cells; binding to bacterial toxins and enzymes of invasion and aggression to neutralize their pathogenic effect.
- ***Opsonisation*** - binding to various cell types: phagocytes, lymphocytes, mast cells, etc. which have receptors that bind Ig. This binding can activate the cells.
- ***AMCT -*** binding to lymphocytes (NK cells) activates their cytotoxicity.
- ***Fixation of complement*** - results in lysis of cells and release of biologically active molecules.
- ***Maternal antibodies*** provide immunity to the fetus and newborn: IgG can bind to receptors on placental trophoblasts, which results in transfer of the Ig across the placenta.

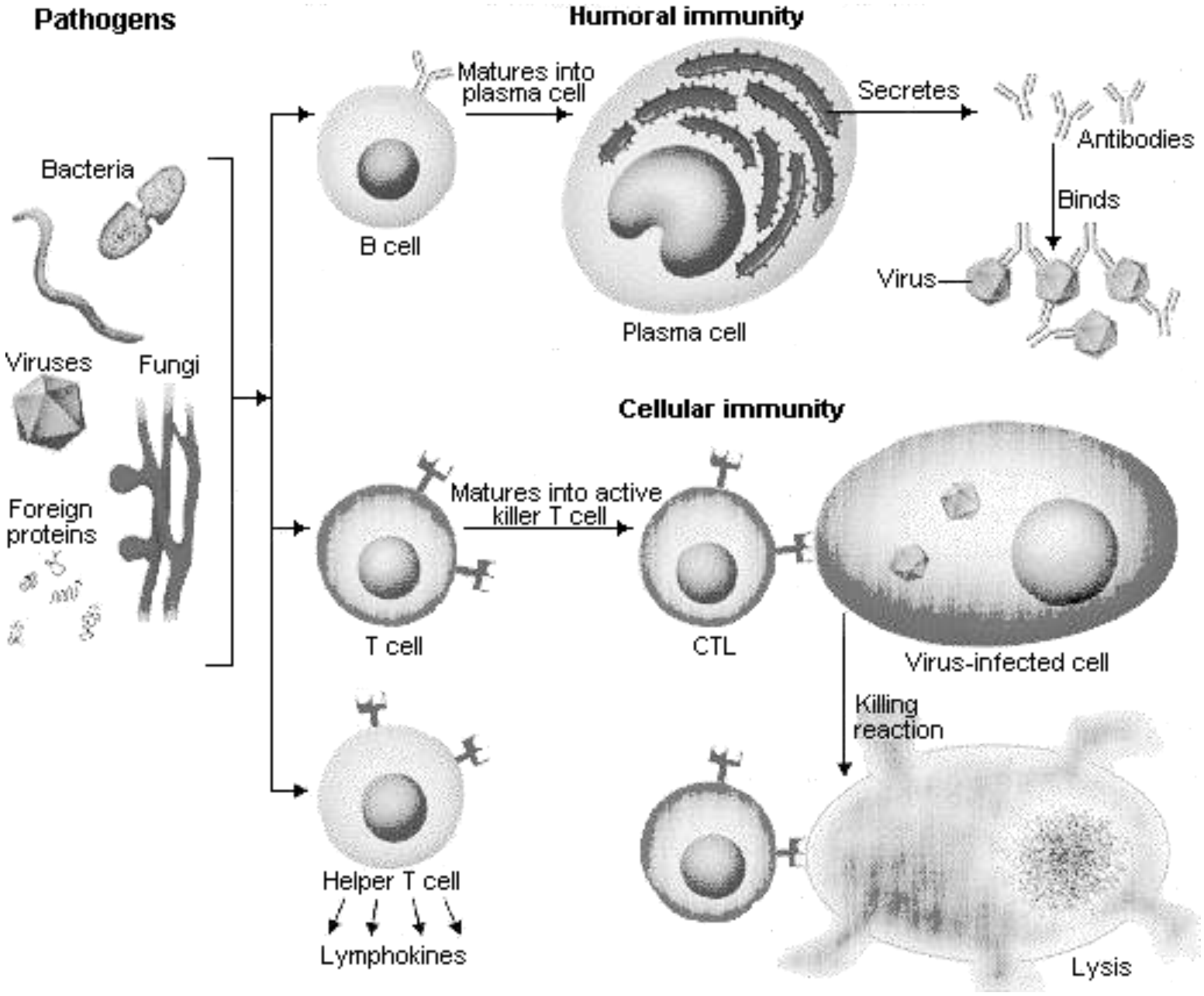
The general scheme of immune response



The general scheme of immune response

- B cells and T cells recognize different antigens and in a different form.
- The B cell uses cell surface-bound Ig as a receptor.
- B cells recognize the following Ags in soluble form:

  1) proteins
  2) nucleic acids
  3) polysaccharides
  4) some lipids
  5) small chemicals (haptens)
- The majority of Ags for T cells are proteins.
- The protein must be fragmented and T cell recognizes it in association with MHC (I or II) expressed on the surface of the target cells or APC.
- To be presented to T cell the protein Ag should be not in soluble form.

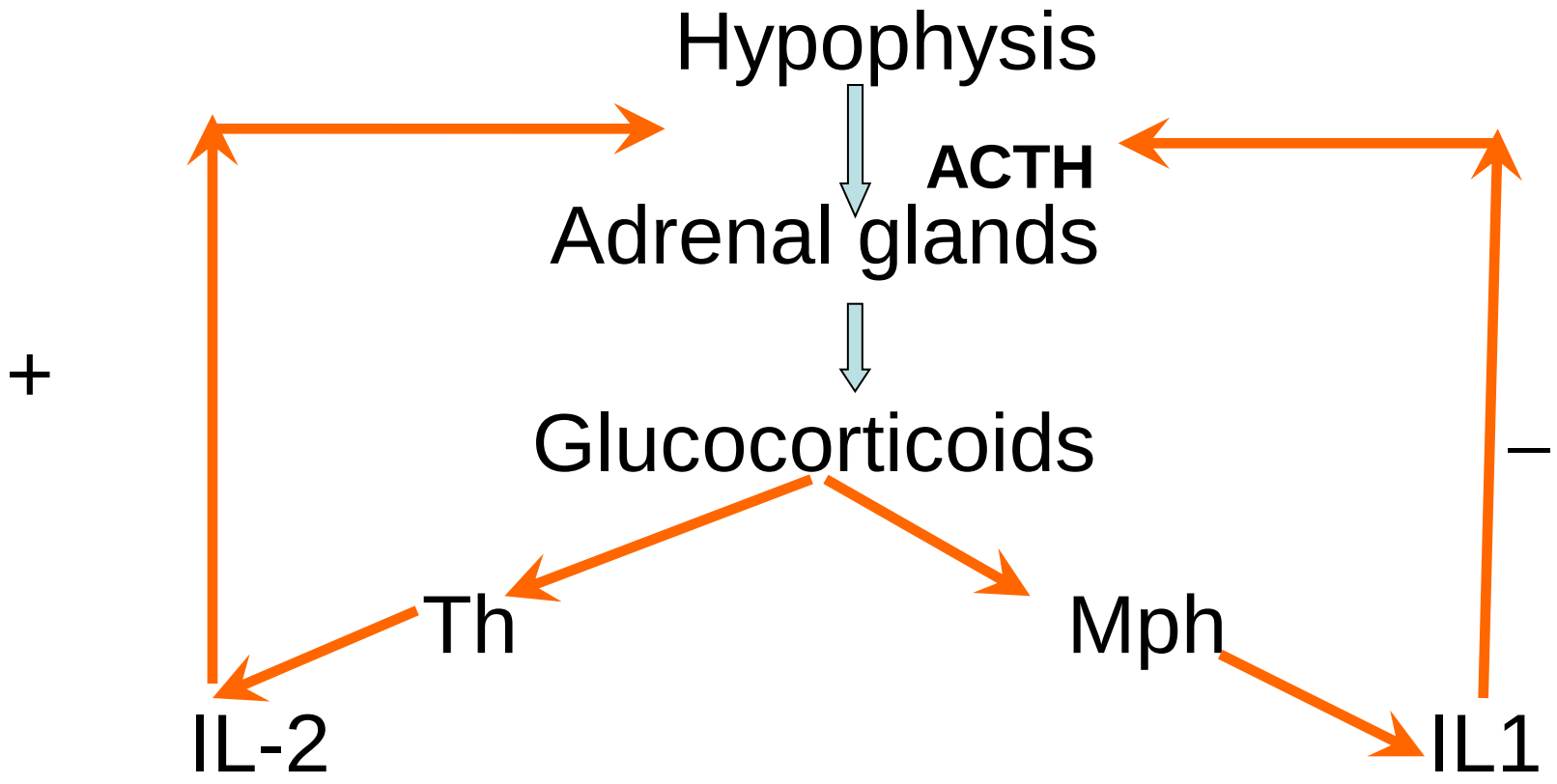

Pathogens
Humoral immunity
Matures into plasma cell
Secretes
Antibodies
Bacteria
B cell
Plasma cell
Binds
Virus
Viruses
Fungi
Cellular immunity
Foreign proteins
Matures into active killer T cell
T cell
CTL
Virus-infected cell
Killing reaction
Helper T cell
Lymphokines
Lysis

Co-operative mechanism of the immune response and its regulation

- ✓**Co-operation and regulation:**
  - ↳Inside of the immune system (interactions between immunocompetent cells)
  - ↳Inside of the whole organism

Co-operation between different systems of the human organism

- *Theory of immunity by Zdradovsky*

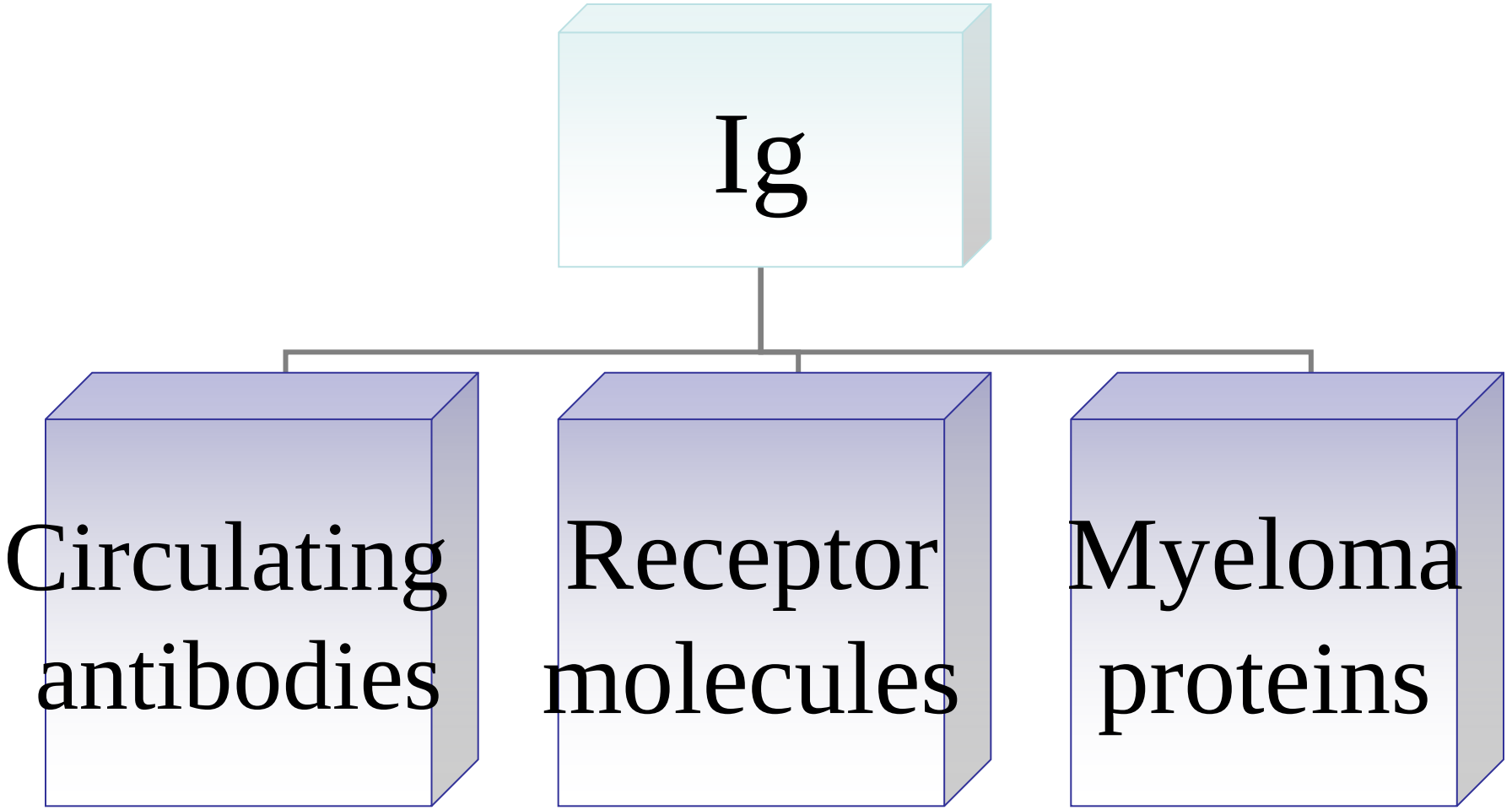


IMMUNOGLOBULINS

Definition of the term

- glycoprotein molecules that are produced by plasma cells in response to an immunogen (antigen)
- the immunoglobulins (Ig) derive their name from the finding that they migrate with globular proteins when antibody-containing serum is placed in an electrical field
- Ig function as antibodies: they are able to bind specifically to one or a few closely related antigens
- each Ig actually binds to a specific antigenic determinant
- antigen binding by antibodies is the primary function of antibodies and can result in protection of the host

Immunoglobulins (existing forms of immunoglobulins)



Circulating antibodies

- *Serum Ig*

  found in blood serum

- *Secretory Ig*

  occur in secretions of mucous membranes and called secretory *IgA - IgAs*

Receptor molecules

- *Ag*-binding receptor of immature B-cells – **IgM**
- *Ag*-binding receptor of mature B-cells – **IgD**

Bence-Jones (myeloma) proteins

- ***Bence-Jones proteins***

  light chains of *Ig*, which synthesized in large amounts in myeloma (Bence-Jones proteinemia).

Basic structure of immunoglobulins: domain and paratope

Three dimensional images of the immunoglobulin molecule show that it is not straight and is folded into globular regions. These regions are called domains.

Domain

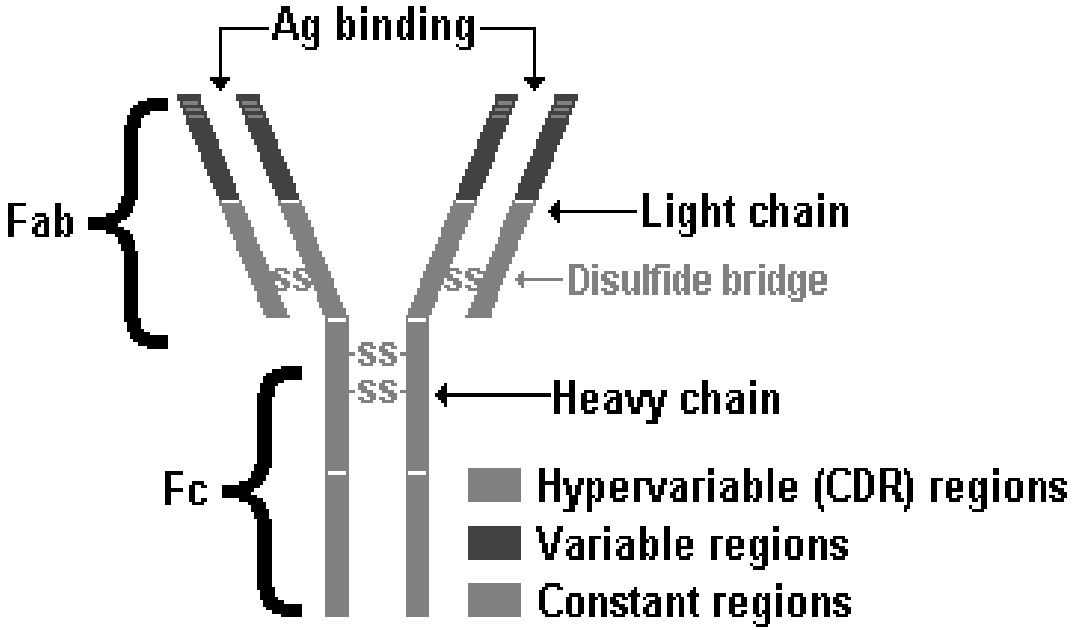
- fragment of polypeptide chain having globular conformation;
- includes about 110 amino acid residues;
- stabilized by disulphide bonds;
- domains connected between each other by linear fragments of polypeptide chain.

Paratope

- antigen-binding centre
- active centre

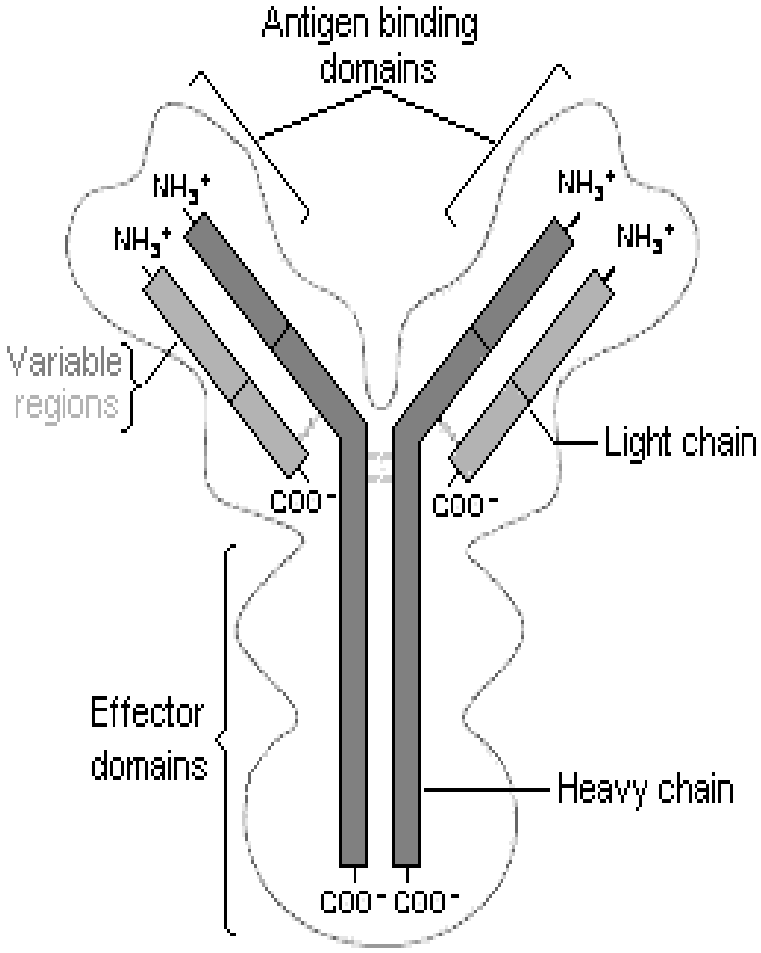
Basic structure of immunoglobulins: heavy and light chains

- All immunoglobulins have a four chain structure as their basic unit.
- They are composed of two identical light chains (L) (23kD) and two identical heavy chains (H) (50-70kD).
- The heavy and light chains and the two heavy chains are held together by inter-chain ***disulfide bonds*** and by non-covalent interactions.

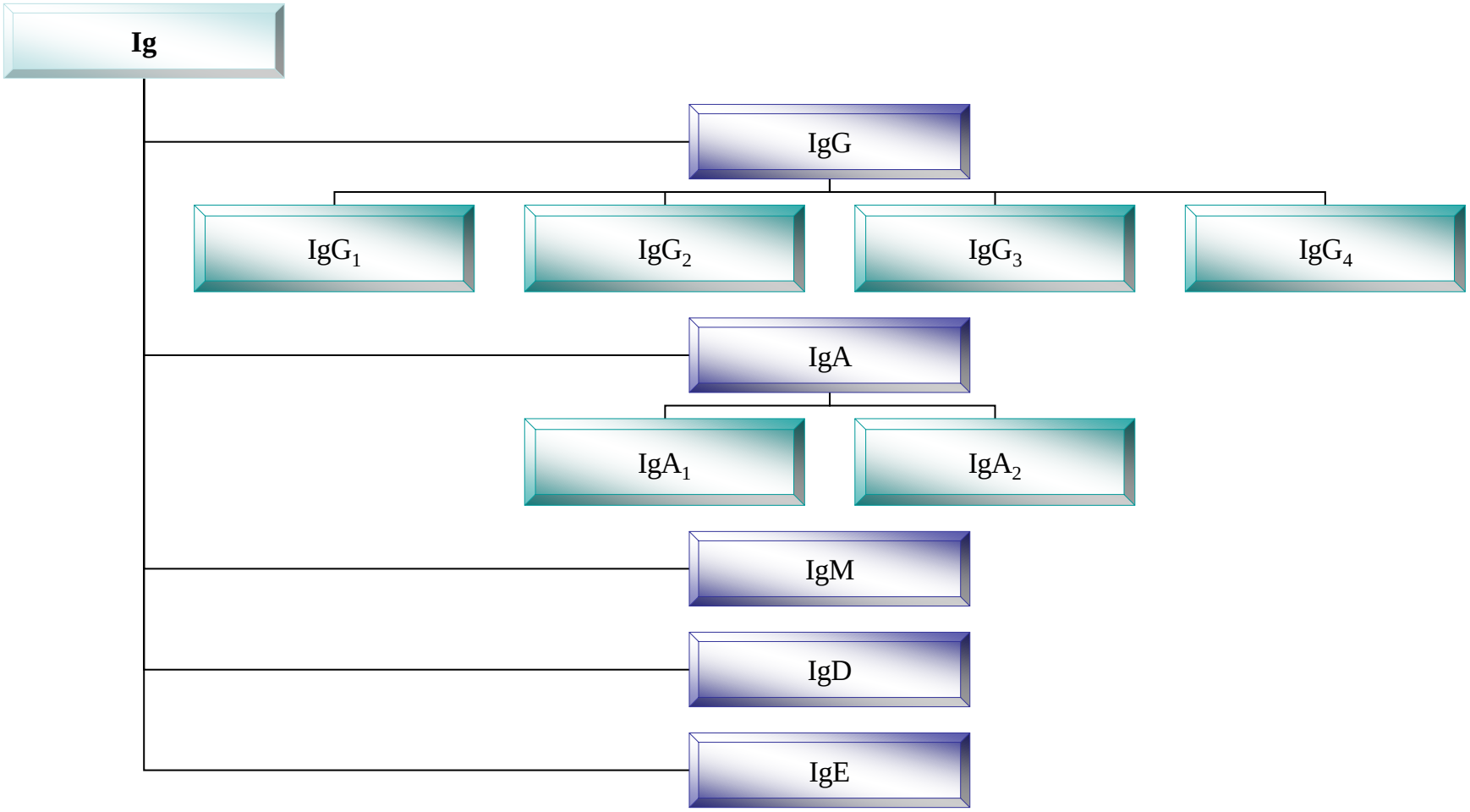


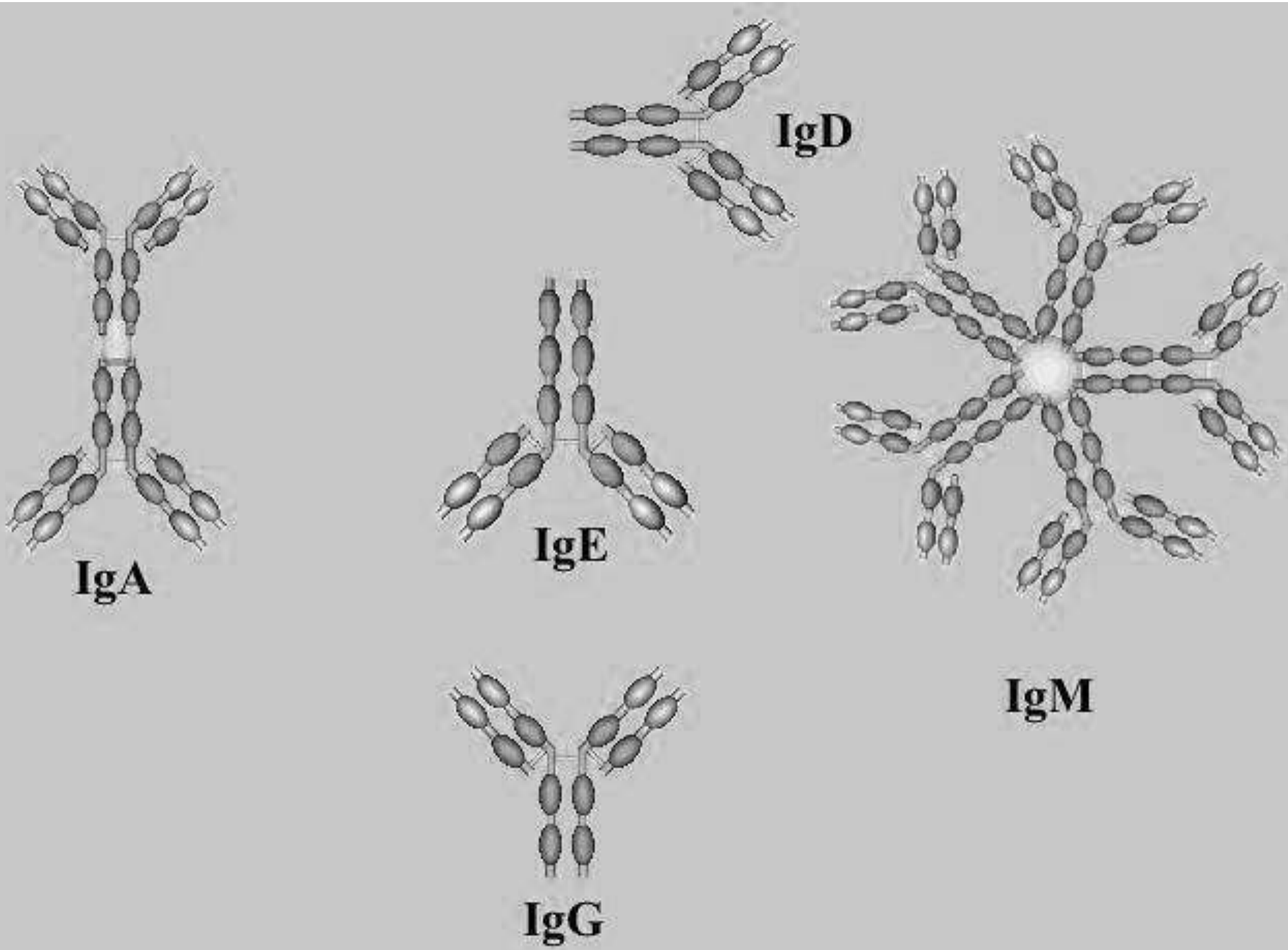
Basic structure of immunoglobulins: variable (V) and constant (C) regions

- Heavy and light chain could be divided into two regions based on variability in the amino acid sequences: **variable (V)** and **constant (C).**
- Two amino-terminal ends - variable regions of the light and heavy chains called **Fab** (antigen binding fragment). It binds antigen.
- The single carboxyl-terminal end called **Fc region** (Fc - crystallizing fragment - easily crystallized). It binds various types of cells (phagocytes, etc) or to C1q fraction of complement.
- **Each Fab fragment is monovalent whereas the original molecule is divalent because it can attach two antigenic determinants in two Fab regions**.



CLASSIFICATION OF IMUNOGLOBULINS:
Immunoglobulin Subclasses




IgD
IgA
IgE
IgM
IgG

Additional polypeptide chains

- *J-chain*

  Unites monomers forming polymeric molecules in IgM and IgAs

- *S-protein* (secretory component)

  Protects of IgAs against proteolysis in the secretions of mucous membranes. Unlike the remainder of the IgA which is made in the plasma cell, the secretory piece is made in epithelial cells and is added to the IgA as it passes into the secretions.

- *M-protein*

  Anchoring of receptor Ig at the membrane of B-lymphocyte

Main functions of antibodies: IgG and IgM

Ig G → secondary immune response.

a) it is the major Ig in serum (75% of serum Ig) and in extra vascular spaces,

b) placental transfer - ***IgG is the only class of Ig that crosses the placenta.***

c) fixes complement ,

d) it is a good opsonin

Ig M→ primary immune response:

a) it is the first Ig to be made by the fetus

b) it is the first Ig to be made by a virgin B-cells when it is stimulated by antigen.

c) as a consequence of its pentameric structure, IgM is a good complement fixing Ig and a good agglutinating Ig

d) B-cell surface Ig-receptors for binding Ag

Main functions of antibodies:

IgA and IgE

Ig A → serum IgA is a monomer but IgAs found in secretions is a dimer.

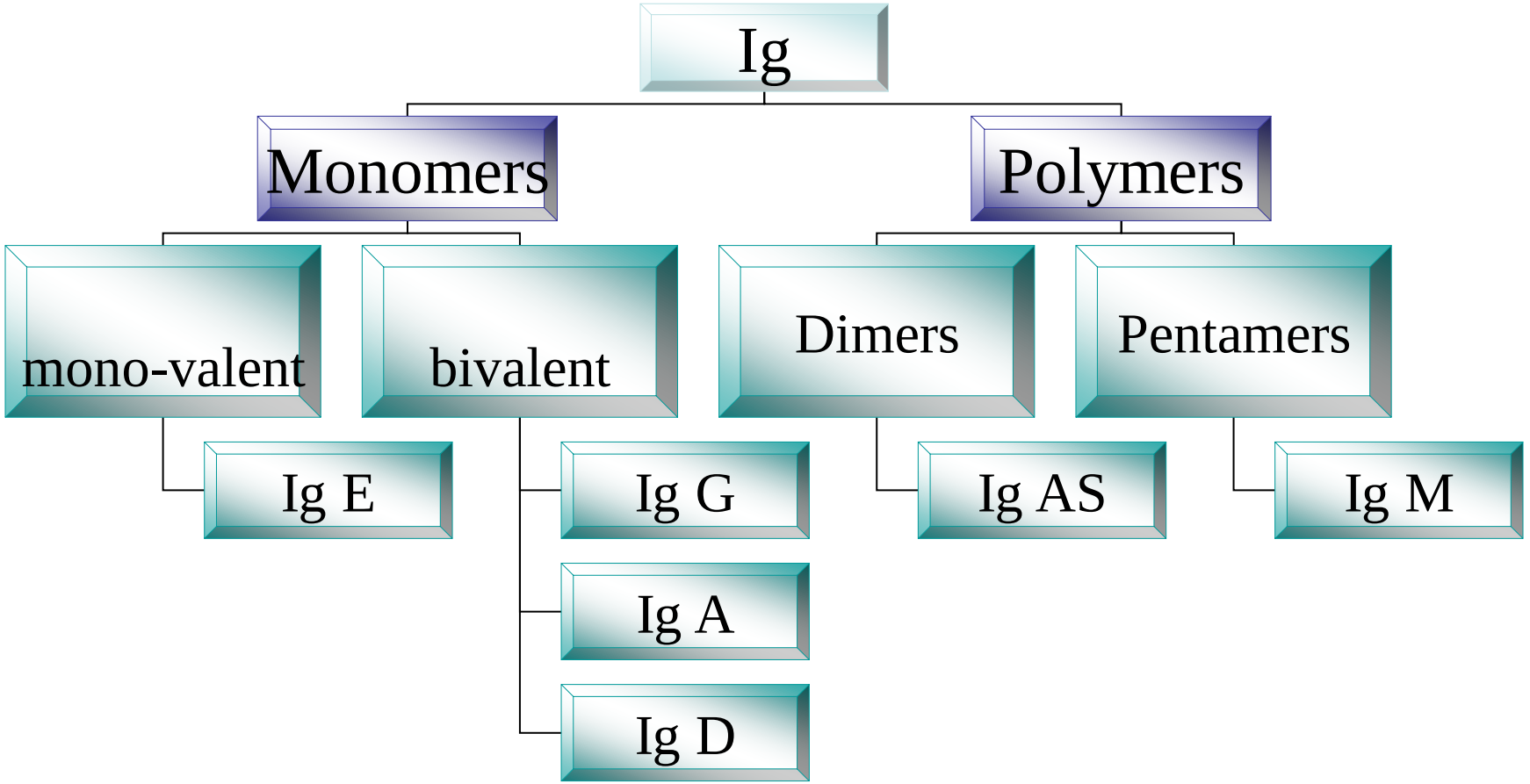
IgAs ↛ immune defense (protection) across mucous membranes.

Ig D →Ag – binding receptor of mature B-cells.

Ig E →anaphylaxis (hypersensitivity): it binds to basophils and is involved in allergic reactions.

IgE also plays a role in parasitic helminth diseases.

Morphofunctional peculiarities of immunoglobulins



Main characteristics of the antigen – antibody complex

- ***AFFINITY*** –is the strength of the reaction between a single antigenic determinant and a single binding site on the antibody: between paratope/epitope. It is the highest in monoclonal antibodies.
- ***AVIDITY*** – avidity is a measure of the overall strength of binding between an antigen and antibodies, shows the stability of the complex between the Ig molecule an antigen as a whole. Avidity is influenced by both the valence of the antibody and the valence of the antigen. The highest avidity is found in IgM.

Normally occurring antibodies

Definition of the term

- ground level of immunoglobulins which are normally formed in human organism without obvious stimulation by antigen

Main function

- display the readiness of macro-organism to immune response
- may indicate the contact with antigen in the far past

Monoclonal antibodies

- ✓***Definition of the term*** - antibodies produced by one clone of plasma cells.
- ✓***The main significance*** – high specificity of the interaction with antigen

INCOMPLETE ANTIBODIES

Definition of the term:

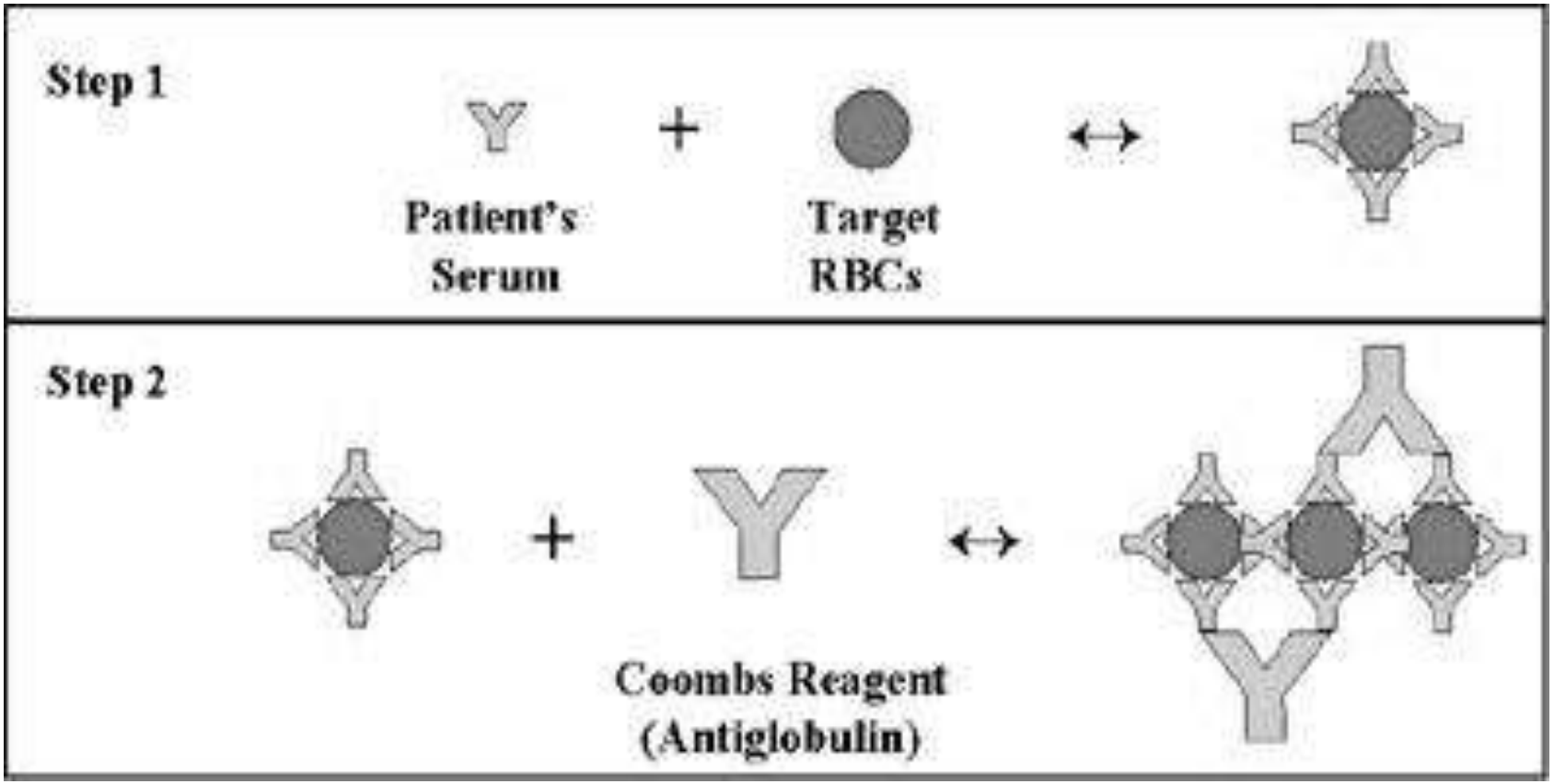
- monovalent antibodies which can't form the lattice (giant immune complexes – precipitates or agglutinates); visible by unaided eye
- blocking antibodies or, reaginic antibodies.

Tests revealing incomplete antibodies

Coombs test - antiglobulin test

1. Incomplete (nonagglutinating) antibody + antigen = no visible reaction
2. + antiglobulin serum (obtained by immunizing of the rabbit with human Ig); the serum contains complete antibodies and reacts with any human immunoglobulins including incomplete antibodies) **=** visible reaction

Coombs test


Step 1
Patient's Serum
Target RBCs
Step 2
Coombs Reagent (Antiglobulin)

ABSYMS

Antibodies which play a role of catalysts in biochemical reactions.

CLONAL SELECTION THEORY (BURNETT'S THEORY)

CLONAL SELECTION THEORY OF PRODUCTION OF ANTIBODIES

MAIN POSTULATES:

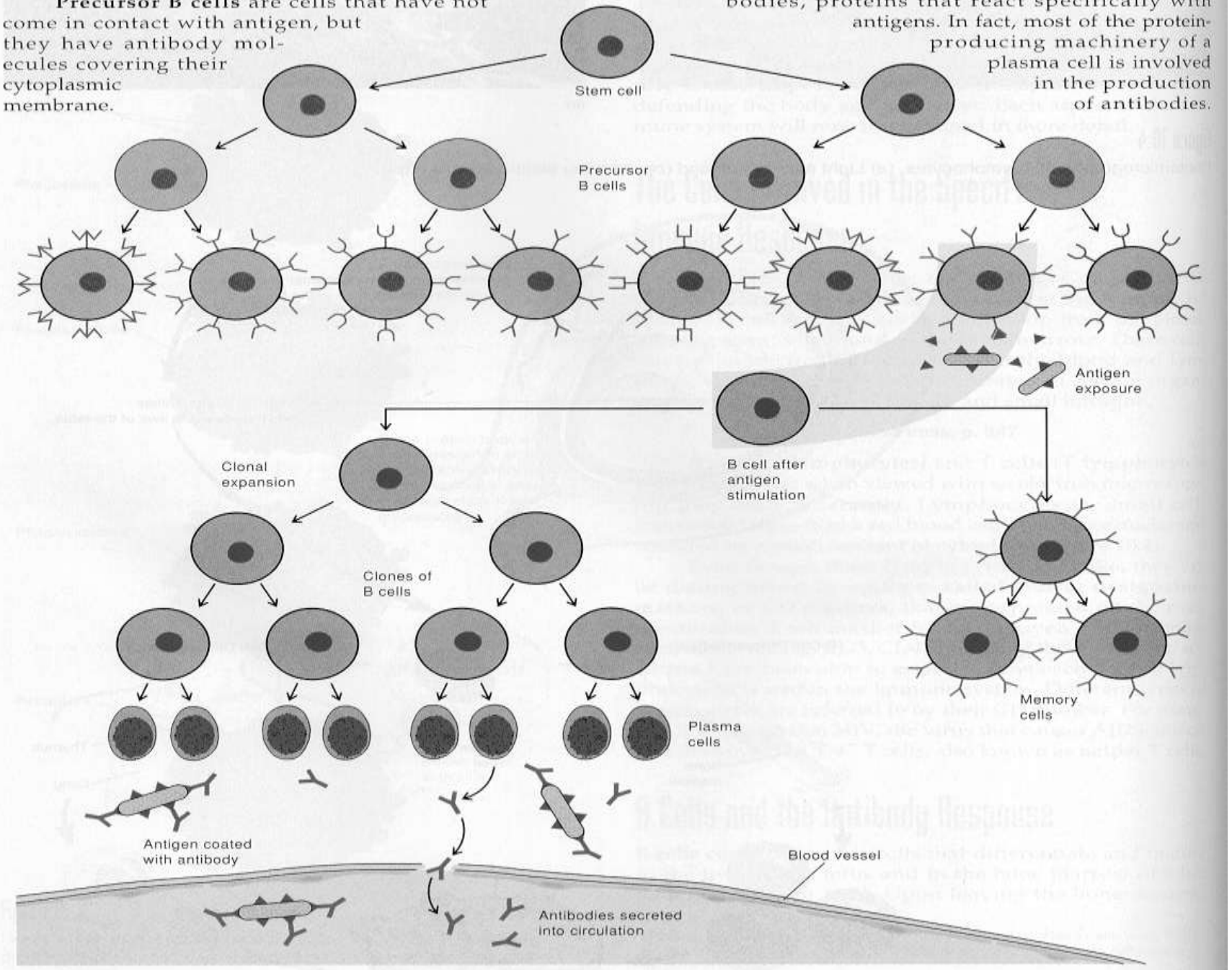
1. Each B-lymphocyte has an antigen specific Ag-recognizing receptors on its surface.
2. In human organism always exist clones of B-lymphocytes having Ag-recognizing receptors which are specific to all possible variants of antigens (each lymphocyte has the genetic information to synthesize antibody molecule as a response to the stimulation by one specific antigen).

CLONAL SELECTION THEORY OF PRODUCTION OF ANTIBODIES

3. The reaction between particular antigen and surface receptor of B-lymphocyte stimulates B-lymphocyte to proliferate into clone *(«EXPANSION OF THE CLONE»)* and to differentiate the lymphocyte to form plasma cell.
4. Plasma cell secretes antibodies which have antigen – binding receptors complementary only to the particular antigen which stimulated expansion of the clone.

Precursor B cells are cells that have not come in contact with antigen, but they have antibody molecules covering their cytoplasmic membrane.

bodies, proteins that react specifically with antigens. In fact, most of the protein-producing machinery of a plasma cell is involved in the production of antibodies.



IMMUNOLOGICAL TOLERANCE TO AUTOANTIGENS
(explanation of the phenomenon according to the clonal selection theory)

- Immunocompetent cells (ICC) which had the contact with the particular antigen in the embryonal period are eliminated → the clones of ICC to the autoantigens are not present in the organism of newborn → the immune response to the autoantigens is not possible.

DIAGNOSTIC SERA

DIAGNOSTIC SERA: the ways of use

- For identification of pathogenic microorganisms

The approaches to production of the diagnostic sera

MICROORGANISM

⇓

IMMUNISATION OF THE ANIMAL

(frequently - rabbit)

⇓

NATIVE SERUM

(polyvalent containing specific and nonspecific antibodies)

⇓

DEPLITION (adsorption) OF THE SERUM ***by Castelljani***

⇓

ADSORPED SERUM

(contains only specific antibodies)

Polyvalent or monovalent (monoreceptor) sera

ALLERGY

Theme № 14

ALLERGY (HYPERSENSITIVITY) – GENERAL DISCRIPTION

- **unusually strong immune response to antigen**
- **undesirable pathological (sometimes fatal) reactions produced by human immune system**
- **hypersensitivity reactions require a pre-sensitized state of the host : the symptons are developing after the second contact with Ag**
- **antigen that stimulates allergic reaction is called *ALLERGEN* *Ag ⇨ Al***
- **the word allergy derives from the Greek words *allos* meaning "other" and *ergon* meaning "work"**

Classification of allergic reactions

- **Classification is based on the mechanisms involved and time taken for the reaction.**
- **Usually hypersensitivity reactions are divided into four types:**

1. Type I hypersensitivity is also known as anaphylactic hypersensitivity.

2. Type II hypersensitivity is known as cytotoxic hypersensitivity

3.Type III hypersensitivity is known as immune complex hypersensitivity.

4. Type IV hypersensitivity is known as cell mediated or delayed type hypersensitivity.

Immediate-type hypersensitivity (ITH)

- **1st, 2nd and 3rd type of allergic reactions are immediate – type hypersensitivity.**
- **The reaction usually takes some minutes to some hours from the time of exposure to the Al.**
- **Immediate-type hypersensitivity reactions are mediated by Ig.**

Delayed-type hypersensitivity (DTH)

- The reaction usually takes 24-48 hours from the time of exposure to the Al.
- This is 4th type of hypersensitivity or cell mediated type.
- Delayed hypersensitivity mediated by T lymphocytes, monocytes and/or macrophages.

The algorithm of the development of allergic reaction

AI

THE FIRST CONTACT WITH IMMUNE SYSTEM

↓

the activation of specific immunocompetent cells

↓

synthesis of specific antibodies (in ITH)

↓

the state called sensitisation

THE SECOND CONTACT WITH IMMUNE SYSTEM

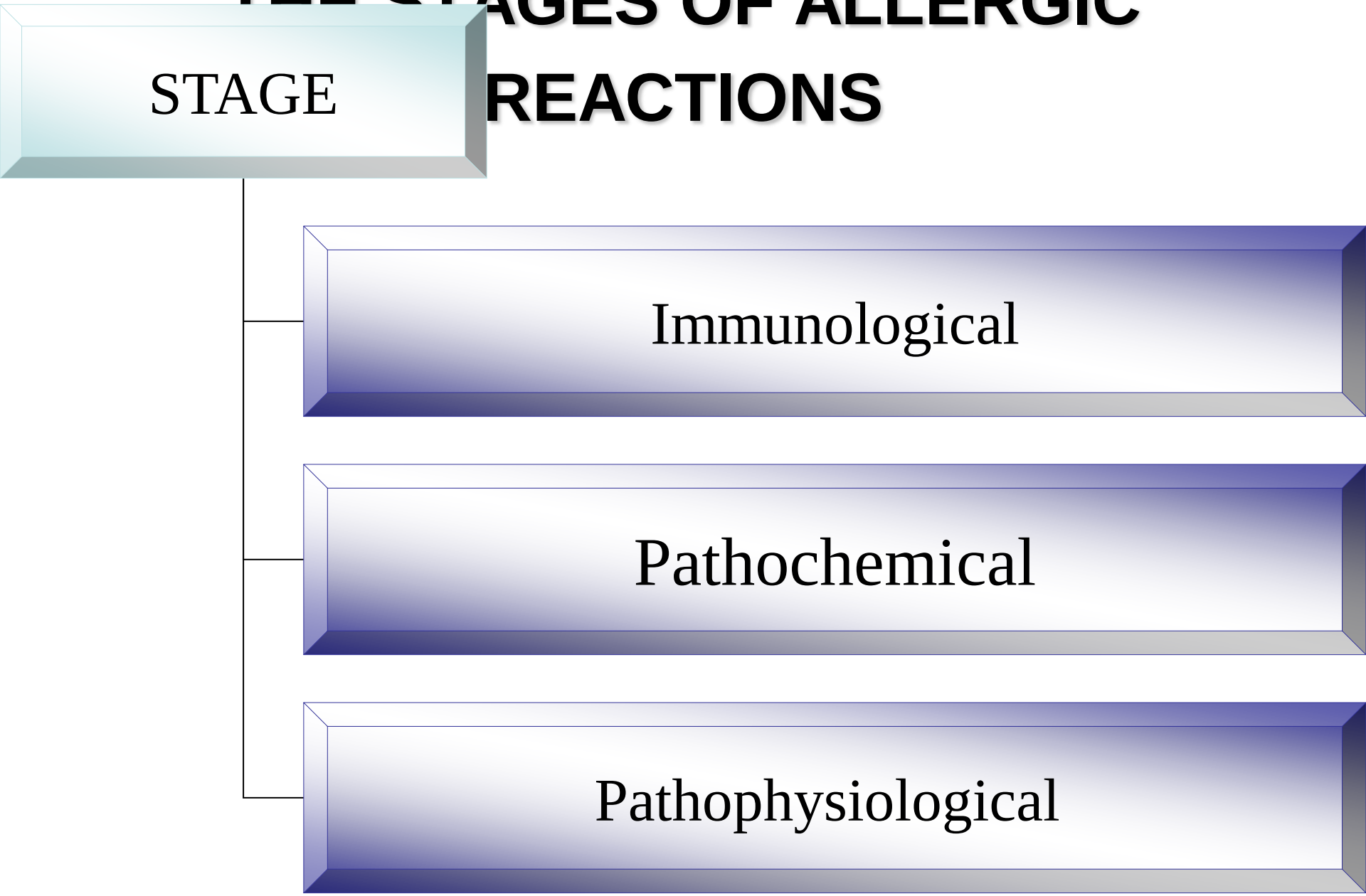
↓

clinical symptoms

↓

visible allergic reaction

THE STAGES OF ALLERGIC REACTIONS



IMMUNOLOGICAL STAGE

- The first contact with an AI.
- Activation of immune cells.
- Synthesis of antibodies (in the case of ITH reactions).
- Synthesis of interleukin-2, interferon-gamma, TNF by activated immune cells (in DTH).

PATHOCHEMICAL STAGE

- activated mast cells and basophiles secrete ***MEDIATORS (histamine, heparin, other inflammatory chemical substances)*** (in ITH)
- activated immune cells secrete **cytokines:**

  IL-2, interferon-gamma, TNF.

PATHOPHYSIOLOGICAL STAGE

- Clinical display of the allergic reaction caused by the effect of mediators of allergy and activated immune cells on the cells of human organism following by the development of specific symptoms:

  rash, itch, swelling, inflammation, tissue damage, etc

ANAPHYLAXIS

(Type I hypersensitivity is also known as immediate or anaphylactic hypersensitivity).

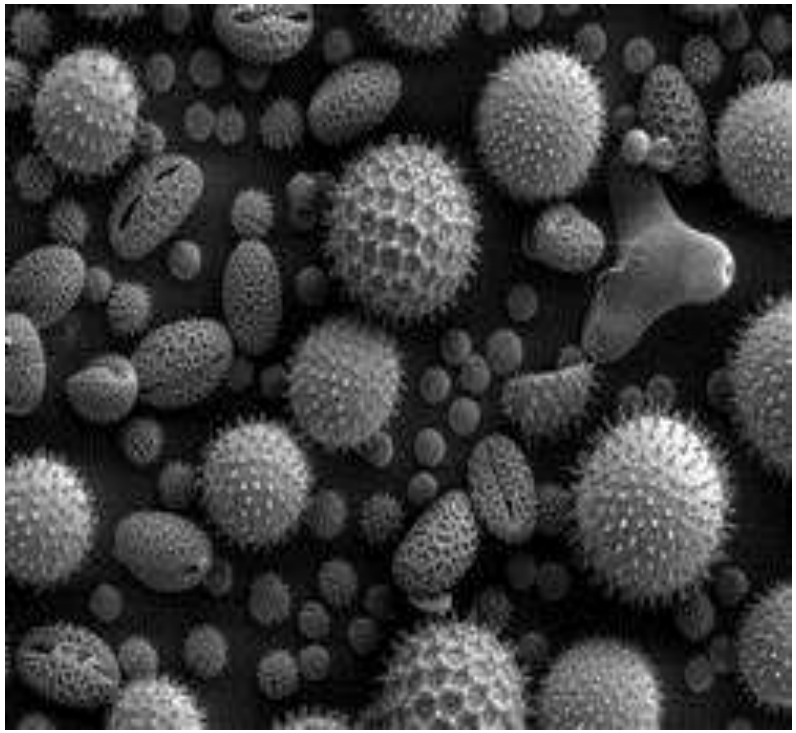
ANAPHYLAXIS

THE ALLERGENS WHICH CAUSE ANAPHYLAXIS

the most frequent Al are :

– *immunologically foreign protein (including vaccines and sera)*

– *antibiotics*



Plant pollens

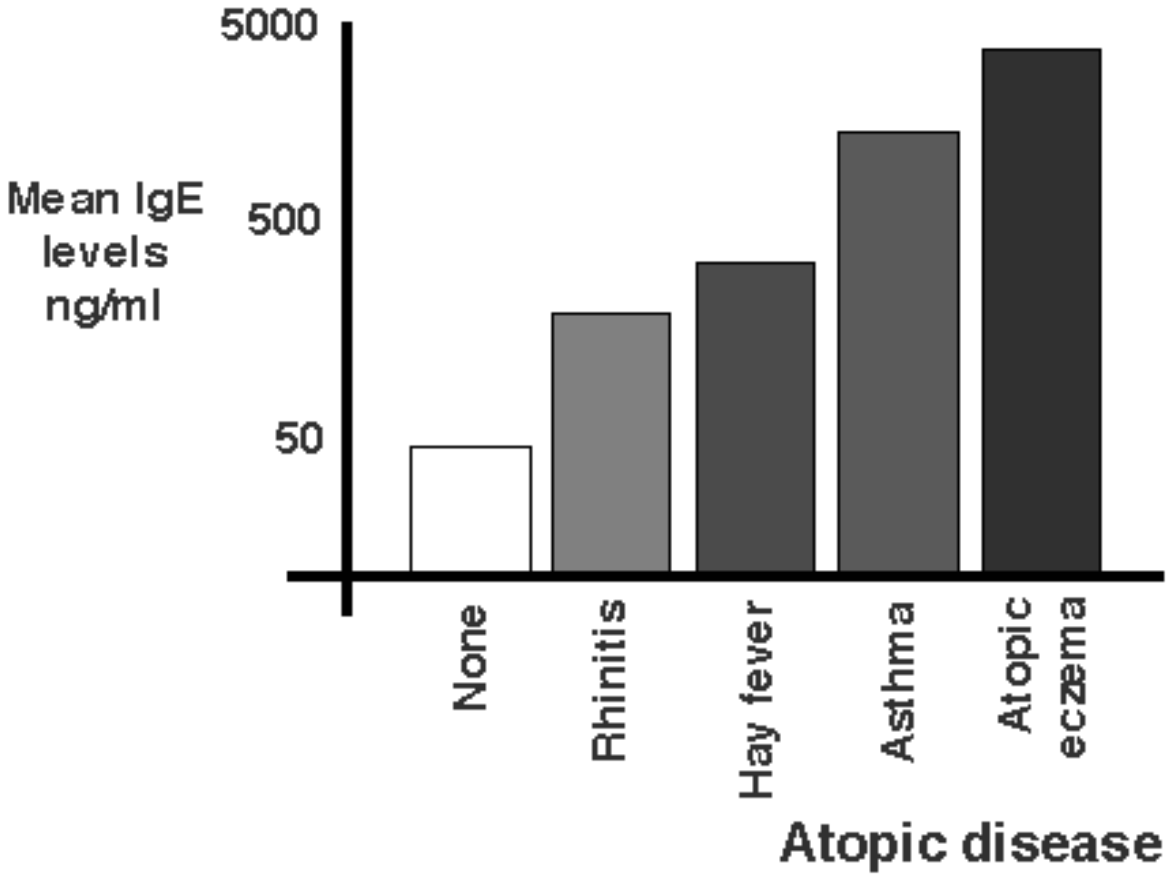
The house dust mite;

its feces and chitin

ANAPHYLAXIS: THE MECHANISM OF THE REACTION

- **In type I hypersensitivity reactions plasma cells secrete IgE as opposed to either IgM or IgG in normal humoral response .**
- **IgE binds to Fc receptors on the surface of mast cells (MC) and basophils (BPH), activates synthesis of mediators which are accumulated in the granules in the cytoplasm of these cells – granulation.**
- **A subsequent exposure to the same Al cross links the cell-bound IgE and triggers the release of mediators – degranulation.**
- **Appearance of clinical symptoms as a result of the affect of mediators on shock organs: asthma, hey fever, anaphylactic shock.**

High IgE levels are asociated with allergic diseases



SHOCK ORGANS

- the organs which are effected when the anaphylactic reaction is developed
- in human – arterioles and bronchi

mediators cause capillary dilation, smooth muscles contraction and airway constriction – the pathological reactions which can lead to systemic anaphylaxis and death

THE SCHEME OF PATHOGENESIS OF I TYPE HYPERSENSITIVITY REACTION

Al

⇩

APC

⇩

B-cell

⇩

Plasma cells

⇩

Ig E

⇩

Receptors of *BPH* or MC

⇩

PROCESS OF GRANULATION–synthesis of mediators

⇩

Sensitisation

Secondary contact with the allergen

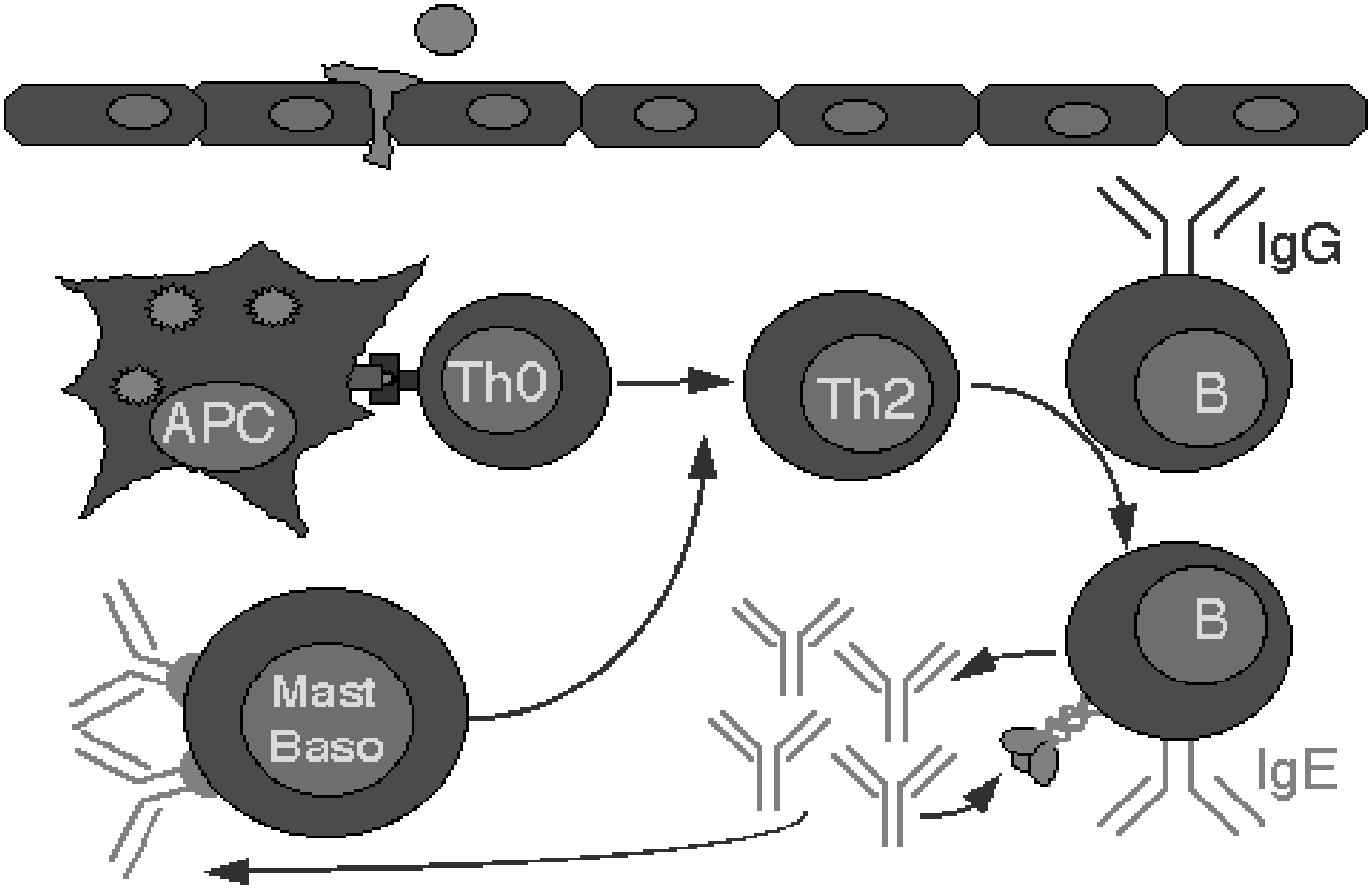
cross-binding of mono-valent *IgE* attached at the membranes of *MC or BPH* by at least bivalent *Al*

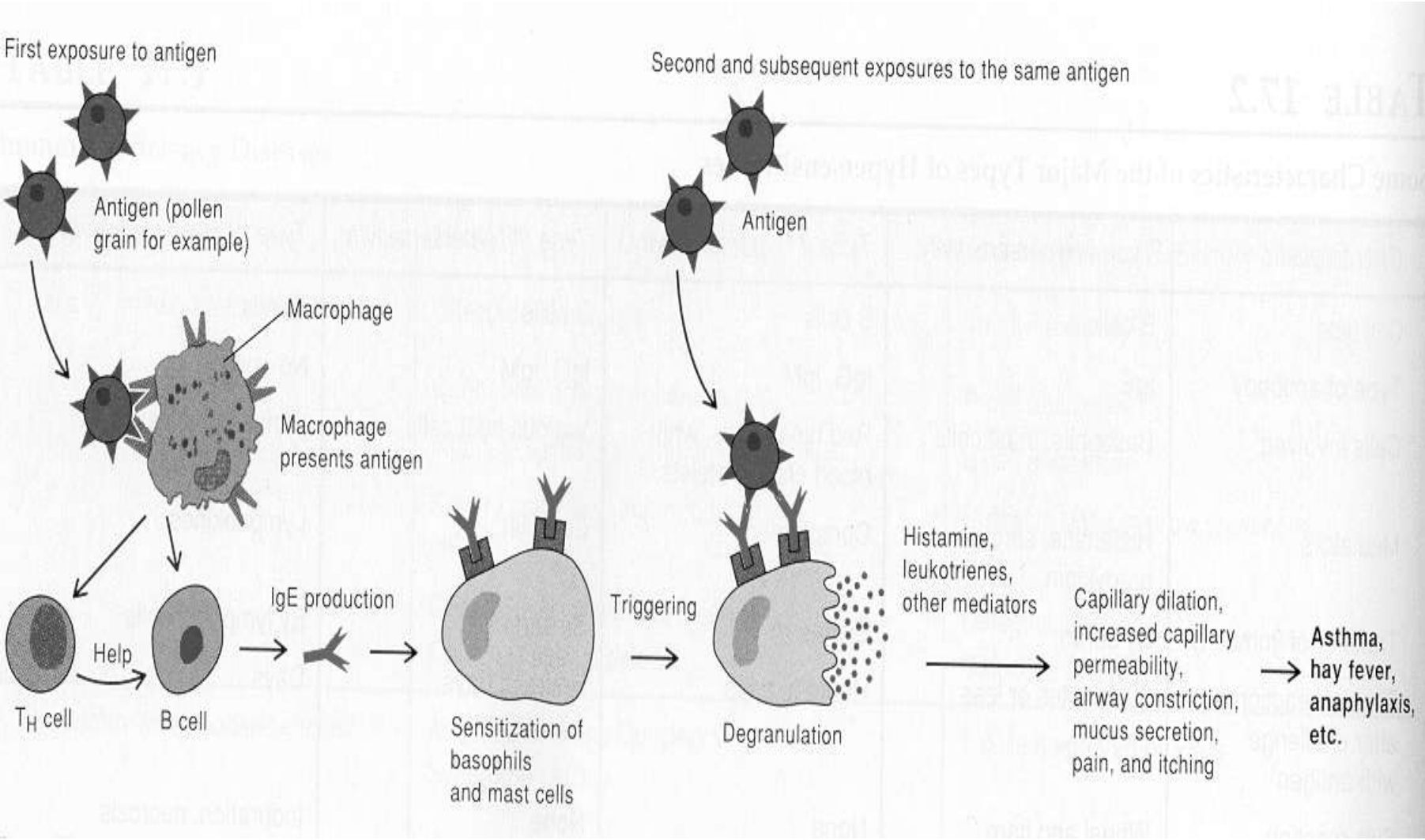
⇩

DEGRANULATION

pathophysiological stage

Allergic immune responses




First exposure to antigen
Antigen (pollen grain for example)
Macrophage
Macrophage presents antigen
Help
T_H cell
B cell
IgE production
Sensitization of basophils and mast cells
Second and subsequent exposures to the same antigen
Antigen
Triggering
Degranulation
Histamine, leukotrienes, other mediators
Capillary dilation, increased capillary permeability, airway constriction, mucus secretion, pain, and itching
Asthma, hay fever, anaphylaxis, etc.

ATHOPY

- inherited predisposition to hyperproduction of IgE as a response to the contact with the antigen which in normal state practically never induces the immune response

The principals of the therapy of anaphylactic reactions

- **If possible, *to avoid* the contacts with the allergen;**
- ***hyposensitization:* the patient is gradually exposed to progressively larger doses of the Al to induce IgG ("the blocking antibody") production, instead of the excessive IgE production;**
- **use of preparations which stabilize the membranes of the mast cells and basophiles,**
- **intravenous injection of monoclonal anti-IgE antibodies,**

The principals of the therapy of anaphylactic reactions

- **use of antagonistic drugs - *antihistamines* to prevent activation of cells and degranulation processes,**
- **use of inhibitors of the late stage of the allergic reaction: *steroid hormones like epinephrine (adrenalin),***
- **<u>the preparation of the emergent help in the case of strong anaphylactic reaction</u> – *adrenaline* (in serious cases – + *prednisolone*).**

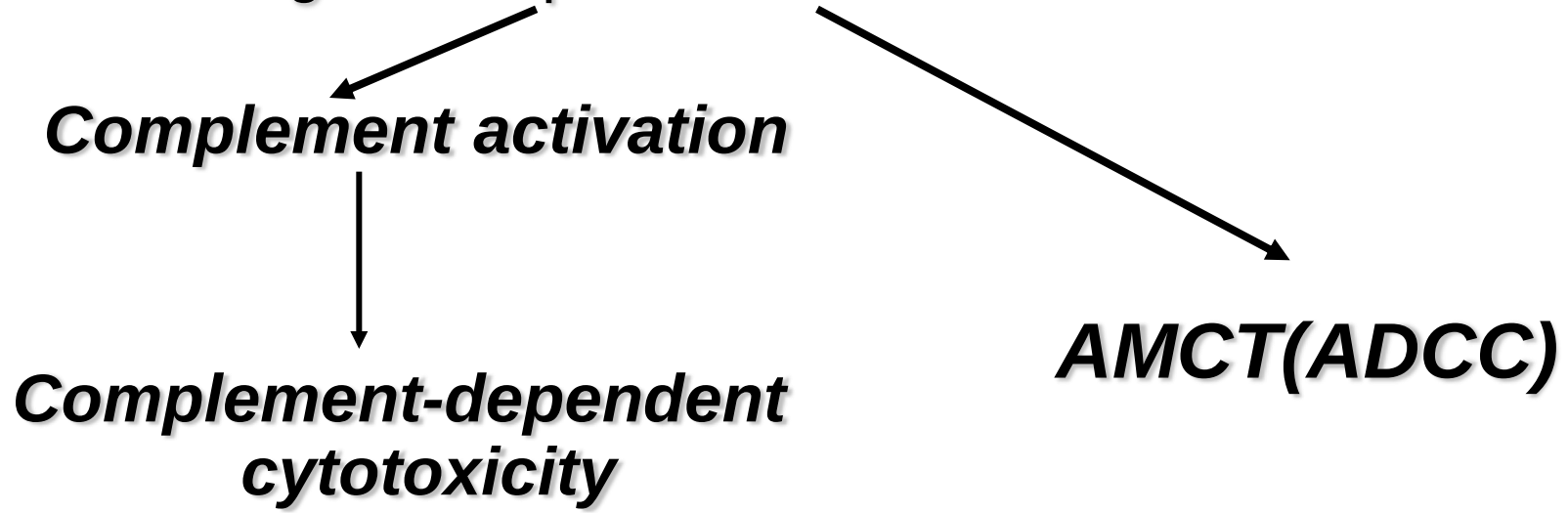
II type allergic reaction

Cytotoxic type of the allergic reaction

Type II hypersensitivity or cytotoxic hypersensitivity.
It affects a variety of organs and tissues.
Al: endogenous antigens or exogenous chemicals (haptens) which can attach to cell membranes.

IgG1-3, IgM
against the surface antigens (self-Ag) of the human cells

against haptens bound to the cell surface



Cytotoxic type of the allergic reaction: main characteristics

- The reaction time is minutes to hours.
- It is primarily mediated by antibodies of the IgM or IgG classes.
- It activates complement.
- Phagocytes and NK cells also play a role in antibody dependent cytotoxicity (ADCC).
- Leads to drug-induced hemolytic anemia, granulocytopenia, thrombocytopenia and other pathologies.

Treatment involves anti-inflammatory and immunosuppressive agents.

Allergic reaction of the III type

(type III hypersensitivity or immune complex hypersensitivity)

Allergic reaction involving immune complexes

- The reaction may take 3 - 10 hours after exposure to the antigen.
- It is mediated by soluble immune complexes.
- The size of immune complexes is important to cause disease and determines the tissue involved:

  in the case of the significant excess of Ag →immune complexes (Ag+Ig) having small or mediate size → deposits of immune complexes in tissues and activation of complement → toxic effect and tissue damage

CLINICAL DISPLAYS

1. ***Inflammation.***
2. ***Damage of the tissues of human organism.***

Treatment includes anti-inflammatory agents

Allergic reaction of the IV type

DTH

symptoms of the allergic reaction appear in

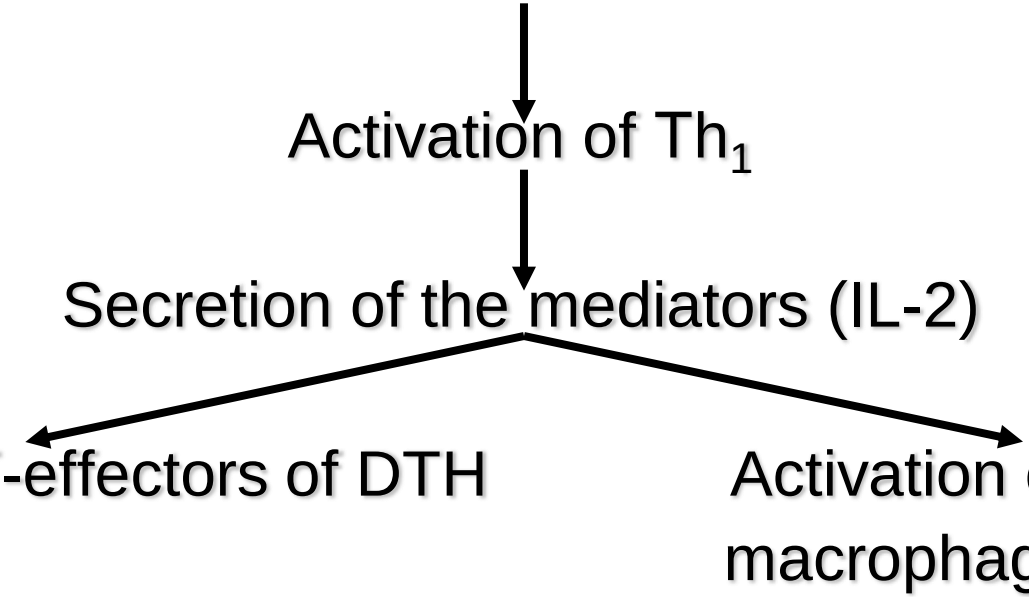
24 – 48 hours

The most frequent allergens are polysaccharides and low molecular weight peptides

AI

low doses

especially in the case of skin testing: tuberculin (Montoux) reaction



Mechanisms of tissue damage in DTH

- Helper T (TH_1) cells - secrete cytokines which activate cytotoxic T cells and recruit and activate monocytes and macrophages.
- Cytotoxic T cells and activated macrophages cause direct damage of tissue.

Corticosteroids and other immunosuppressive agents are used in treatment.

INFECTIOUS ALLERGY

General description

Allergic state developed after the contacts with infectious allergen - *MICROORGANISM*

accompanies infectious process and participates in pathogenesis of the disease

The main cases when DTH can occur

***Infectious diseases which are accompanied by DTH reactions*:**

- chronic bacterial
- viral
- mycosis
- invasions

Diagnostic importance:

skin testing (e.g. tuberculin testing).

ALLERGY CAUSED BY MEDICATION (EFFECT OF MEDICAMENTS)

All four types of hypersensitivity reactions could be involved

Drug-induced hypersensitivity reactions

- Anaphylactic reactions to antibiotics.
- Cytotoxic hypersensitivity: drug-induced hemolytic anemia, etc. The immune response is developed towards hapten + towards protein-carrier = autoantigen.
- DTH.

DIAGNOSTICS OF ALLERGY

Diagnostics of allergic reactions

- ***I type of allergic reaction***skin tests (reading results after *20 min*) + revealing of *IgE*
- ***II type of allergic reaction***

Revealing of ***Ig*** synthesized against blood cells

- ***III type of allergic reaction***

Revealing of the circulating immune complexes (CIC)

- ***IV type of allergic reaction***

Skin tests (reading results after ***24-48 hrs***) + revealing of sensitized lymphocytes and macrophages in vitro.

REACTION OF PRECIPITATION

GENERAL TERMS

- Precipitinogen – an antigen – participant of the RP.
- Precipitin – an antibody - participant of the RP.
- Precipitate – immune complex forming as a result of the RP.

Application of the RP:

1. **diagnostics of infections,**
2. **legal medical expertise:** to determine the origin of proteins when blood spots and other physiological liquids should be analyzed,
3. **food products control**

   revealing of falsified fish and meat.

The procedure of setting up of the RP :

- In test tubes.
- In gel (immunodiffussion):
  - Modern techniques:
    - ✓***IMMUNOELECTROPHORESIS***
    - ✓ ***IMMUNOBLOTTING.***
- Reaction of neutralisation of the toxin by antitoxic serum.

SINGLE IMMUNODIFFUSION

one reactant (precipitating serum) remains fixed

in an polyacrylamide gel

↓

the mixture of antigens is placed

Into a well punched out of gel to let them to diffuse through semisolid medium

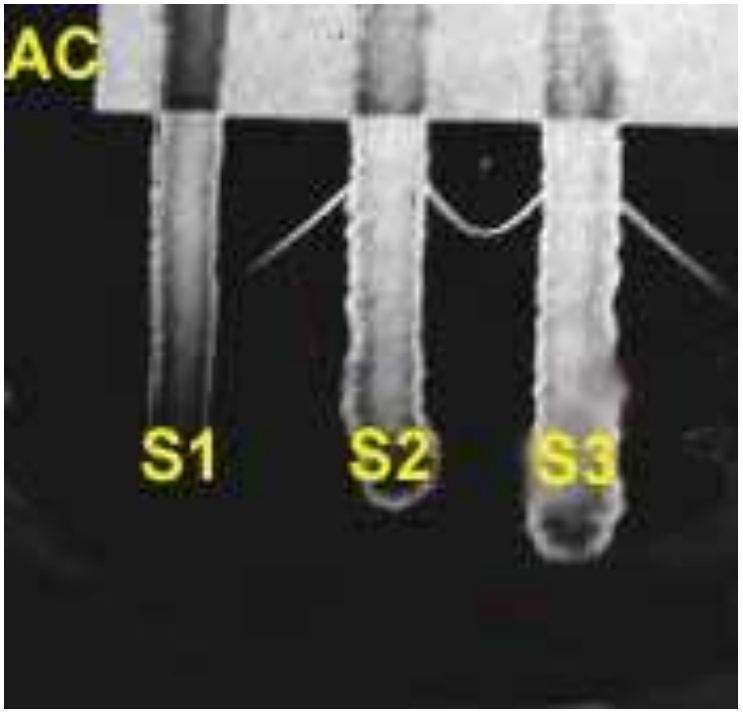
↓

- the lines of precipitate are formed in the place of the antibody – antigen complex formation and the number of lines **=** number of antigens,
- the length of the way measured from the start to the end of the way **=** antigen concentration

Double immunodiffusion (by Ouchterlony)

- both reactants (antigen and antibody) diffuse toward each other and lines of precipitate are formed in the place of the antibody – antigen complex formation .

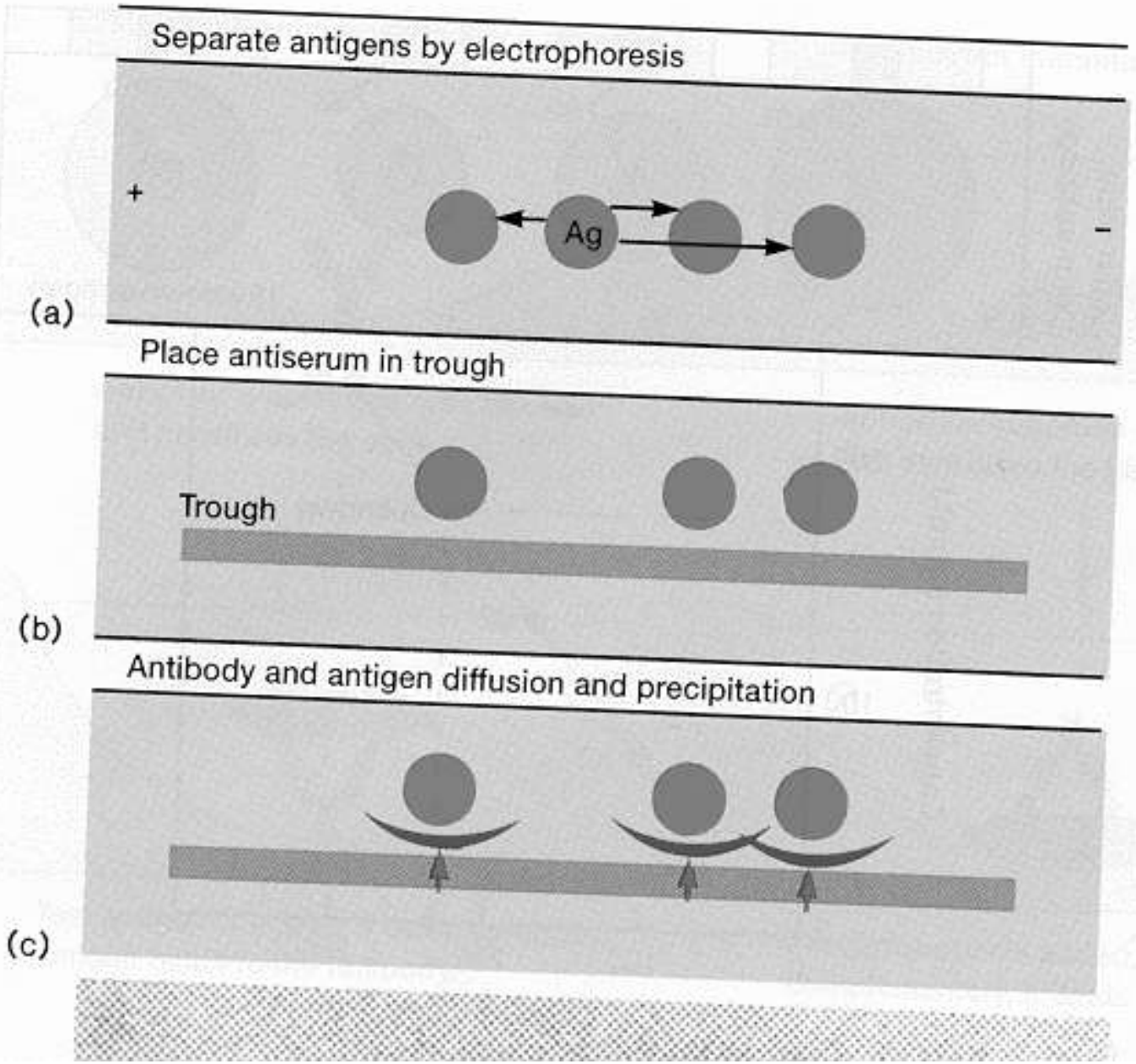

Autoantibodies To
Nuclear Antigens I.D. Plate
Plate No. ____
1
2
3
4
5
6
Immuno Concepts Inc., Sacramento, CA 95827
For Technical Service (800) 251-5115
CA (916) 363-2649 (collect)
Patent Pending


AC
S1
S2
S3

MODERN VARIANTS OF THE RP

IMMUNOELECTROPHORESIS

1. a complex mixture of antigens is placed in a well punched out of an polyacrylamide gel and the antigens are electrophoresed,
2. the antigens are separated according to their mass and charge in electric field,
3. in the trough cut in the gel (is parallel to the track of the protein migration) precipitating serum is placed,
4. as the antibodies diffuse into the gel, precipitin lines are produced in the equivalence zone when an Ag/Ab reaction occurs;
5. appearance of the arch-like lines could be used to analyze the original protein mixture by:
   - ✓ their number,
   - ✓ position,
   - ✓ shape.


Separate antigens by electrophoresis
+
Ag
–
(a)
Place antiserum in trough
Trough
(b)
Antibody and antigen diffusion and precipitation
(c)

IMMUNOBLOTTING

1. electrophoretic separation of protein antigens in polyacrylamide gel,
2. transfer of the proteins to nitrocellulose membrane with use of special equipment – blotting,
3. treatment by monoclonal antibodies,
4. revealing of the precipitates with labeled antiglobulin serum (for example in ELISA).

Reading results of the blotting test

Reaction of neutralisation of the toxin by antitoxic serum (RN)

In vitro neutralisation reaction:

1. in test tubes

- reaction of *flocculation* – the friable precipitate is forming called flocculate

2. in gel

- Ouchterlony's immunodiffussion – for revealing of the toxins produced by corynebacteria which cause diphtheria.

In vivo neutralisation reaction:

toxin + antitoxic serum

↓

injection to sensitive experimental animal

absence of the effect of toxin

↓

Positive RN

visible effect of toxin (death of the animal or necrosis of skin in the place of the injection)

↓

Negative RN

Immunopathology and Clinical Immunology. Immunity in Transplantation and Cancer

Theme N15

Tolerance: general description

Tolerance – is a state of **specific** immunological non-reactivity to an antigen:

- ✓ previous exposure to the same Ag is necessary for appearance of tolerance,
- ✓ it is an active Ag-dependent process,
- ✓ it can exist in T-cells or/and B cells like immunological memory.

Tolerogen - an antigen that induces tolerance.

- ✓ ***all*** antigens can play a role of tolerogens: it is possible to induce tolerance to non-self antigens
- ✓ ***polysaccharide*** antigens are the strongest tolerogens

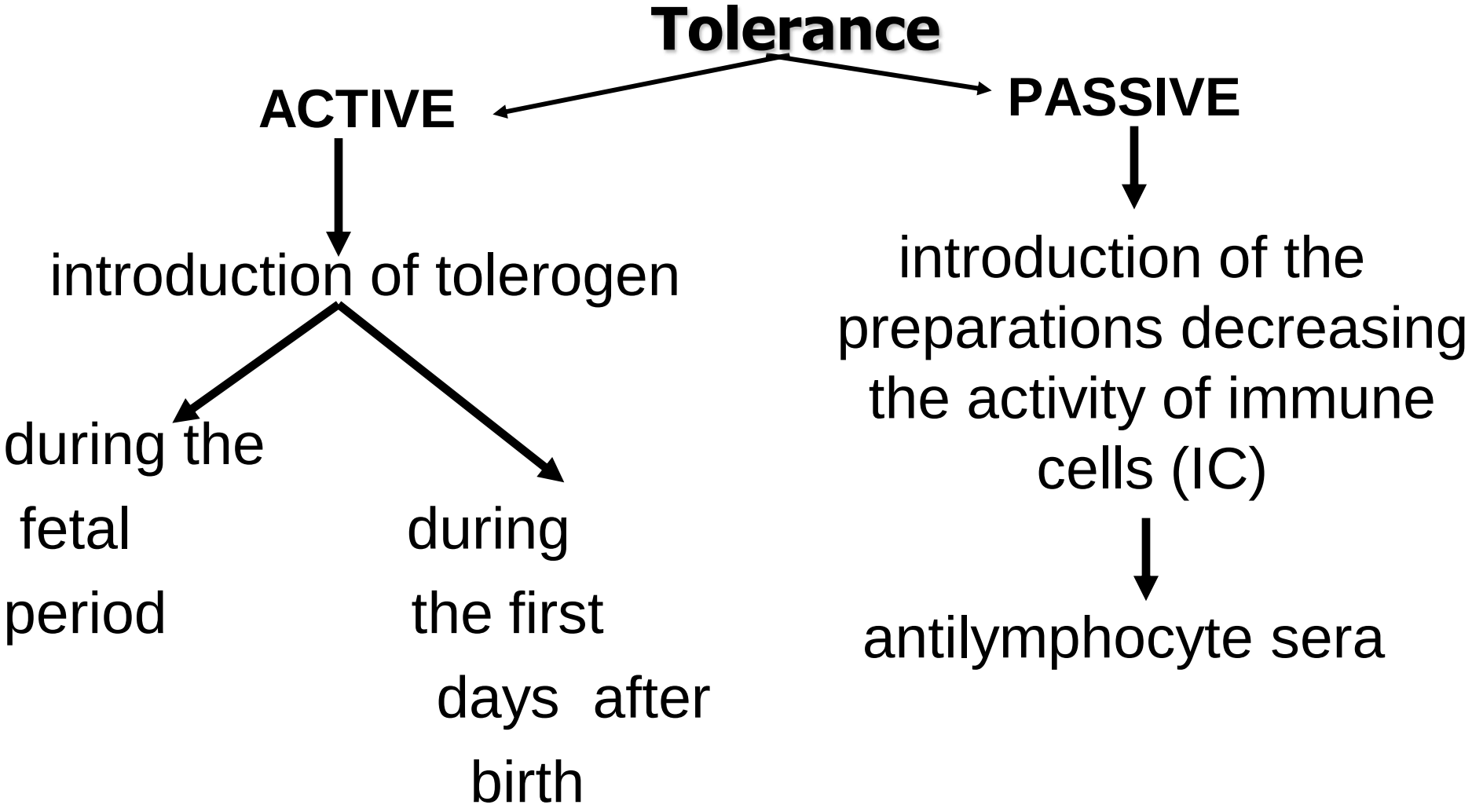
CLASSIFICATION OF TOLERANCE

BY ITS ORIGIN:

- ***Innate*** (naturally occurred)
  - towards one's own tissue antigens (self Ag)

- ***Acquired***
  - active
  - passive

The scheme of the development of acquired tolerance



Factors that determine induction of tolerance	
factors that affect response to Ag	**favor tolerance**
physical form of antigen	soluble and relatively small Ag (not processed by APC)
route of Ag administration	oral or sometimes intravenous
dose of Ag	very large or very small dose
age of responding organism	newborn, immunologically immature
differentiation state of cells	relatively undifferentiated: B cells with only IgM (no IgD) and T cells

The mechanisms of the development of the state of tolerance

1. ***Anti-idiotype antibodies*** - elimination or blocking of Ag-binding receptors in the clones of immune cells produced during the process of tolerization or appeared towards own tissue Ag.
2. ***Suppressor cells -*** T cells induced both at low and high doses of Ag block proliferation or biosynthetic activity in effector IC directly or by production of cytokines.
3. ***Clonal deletion*** - programmed death of IC (auto-reactive T-cells produced towards self Ag).

The mechanisms of termination of tolerance

- **prolonged absence of exposure to the tolerogen,**
- **treatments which severely damage the immune system (x-irradiation),**
- **immunization with cross reactive antigens (autoimmune disease).**

Autoimmunity

Definition of the term

- **breakdown of mechanisms responsible for self tolerance,**
- **induction of immune response towards own tissue's antigens – autoantigens,**
- **abolition of the state of innate tolerance.**

<u>Autoimmunity</u> leads to numerous autoimmune diseases when products of the immune system cause damage to the self.

Autoimmunity

Characteristic features

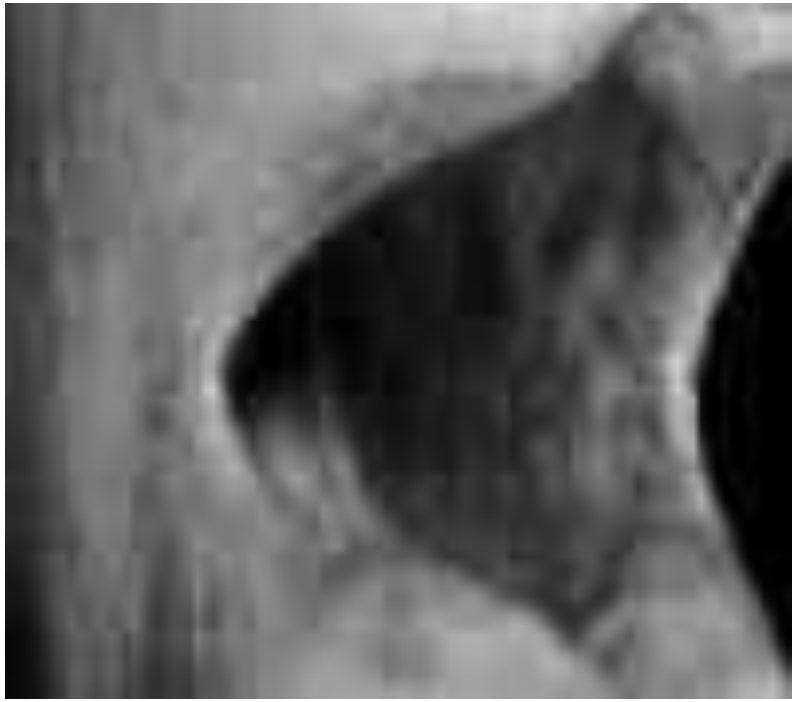
- Both antibodies and effector T cells can be involved in the damage in autoimmune diseases.
- The immune response is directed against antigen(s) associated with the target organ being damaged.
- There is a genetic predisposition for autoimmune diseases in humans: certain association between HLA types and autoimmune diseases has been noted.

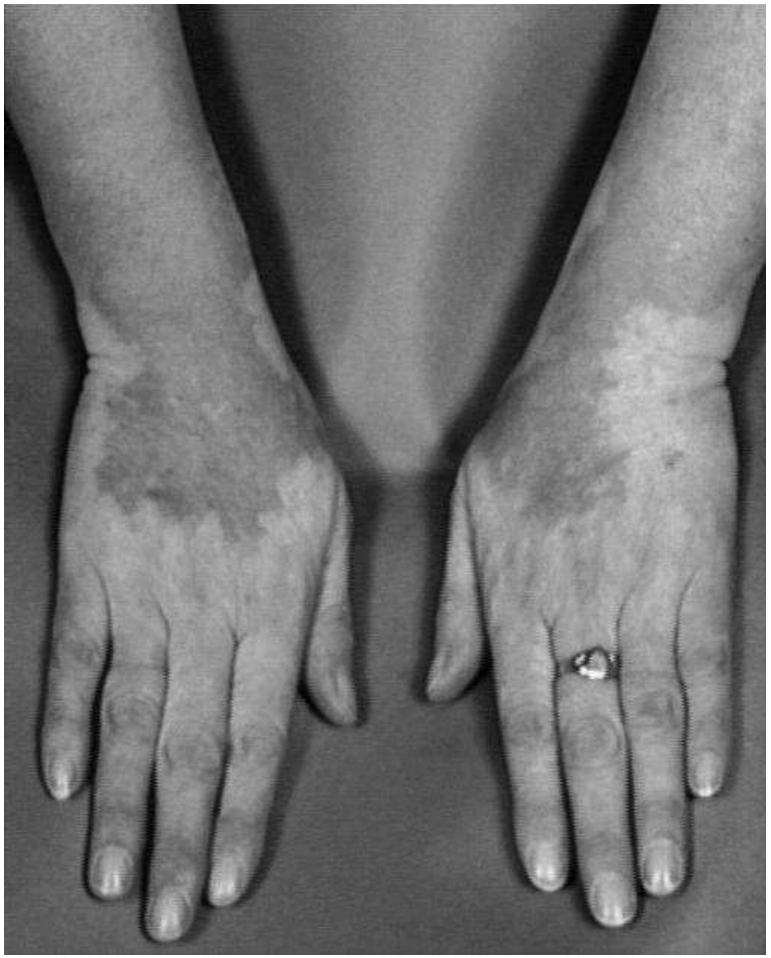
Mechanisms of the development of autoimmune reaction

- **Damage of the surface Ag:** self antigens are subtly altered (in viral infections).
- **Circulating of exogenous cross reactive antigens:** microbial Ag that cross-react with human self Ag (found in chronic infections with persistence of microbe: streptococcal proteins and myocardial tissue Ag in rheumatic fever).
- **Altered self antigens** (effect of chemicals): attachment of foreign substances to the human cells and conversion of these cells into the carriers of the alien epitopes.

- **Accidental traumatic injury or surgery:** release of Ag from the tissues which are normally isolated from immune system (testes, brain, eye, etc).
- **Polyclonal activation of IC** by mitogens (endotoxin).
- **Escape of auto-reactive clones:** the negative selection in the thymus may not be fully functional to eliminate self reactive cells.
- **Regulatory bypass within the immune system:**
  - **the failure of suppressor cells,**
  - **abnormal expression of class II MHC molecules on the surface of cells (thyroid cells in autoimmune - Hashimoto's thyroiditis).**

Autoimmune diseases





Spectrum of autoimmune diseases and target organs

Disease	Organ
Hashimoto's thyroiditis	Thyroid
Hemolytic anemia	Red cells
Addison's disease	Adrenal
Premature onset menopause	Ovary
Male infertility	Sperm
Insulin dependent diabetes	Pancreas
Primary biliary cirrhosis	Liver
Ulcerative colitis	Colon
Rheumatoid arthritis	Skin, kidney, joints etc
Vitiligo	Skin joints

«Immune deficiency» definition of the term

- ***«IMMUNODEFICIENT STATE»:*** disturbance of normal immune status caused by defect of one or several mechanisms of immune response.

Immune deficiencies (classification)

primary (innate)

secondary (acquired)

- **humoral**
  - **deficiency of B-system**
  - **deficiency of complement system**
- **cell-mediated**
  - **deficiency of phagocytosis**
  - **deficiency of T-system**
- **combined**

PRIMARY IMMUNODEFICIENCIES

Primary immunodeficiencies (ID) - inherited defects of the immune system:

- disorders of T cells (congenital thymic aplasia),
- disorders of B lymphocytes (hypoglobulinemia or agammaglobulinemia),
- nonspecific immune systems (defects of the phagocytic system and disorders of complement system).

SECONDARY AND IATROGENIC IMMUNE DEFICIENCIES

- **ID associated with infections (a decrease in the number of Th cells in AIDS),**
- **ID associated with aging (a progressive involution of thymus, a decrease in T cells function),**
- **ID associated with malignancies and other diseases (B cell deficiencies in multiple myeloma, impaired T-cell functions in solid tumors).**

Clinical Immunology

Definition of the term clinical immunology

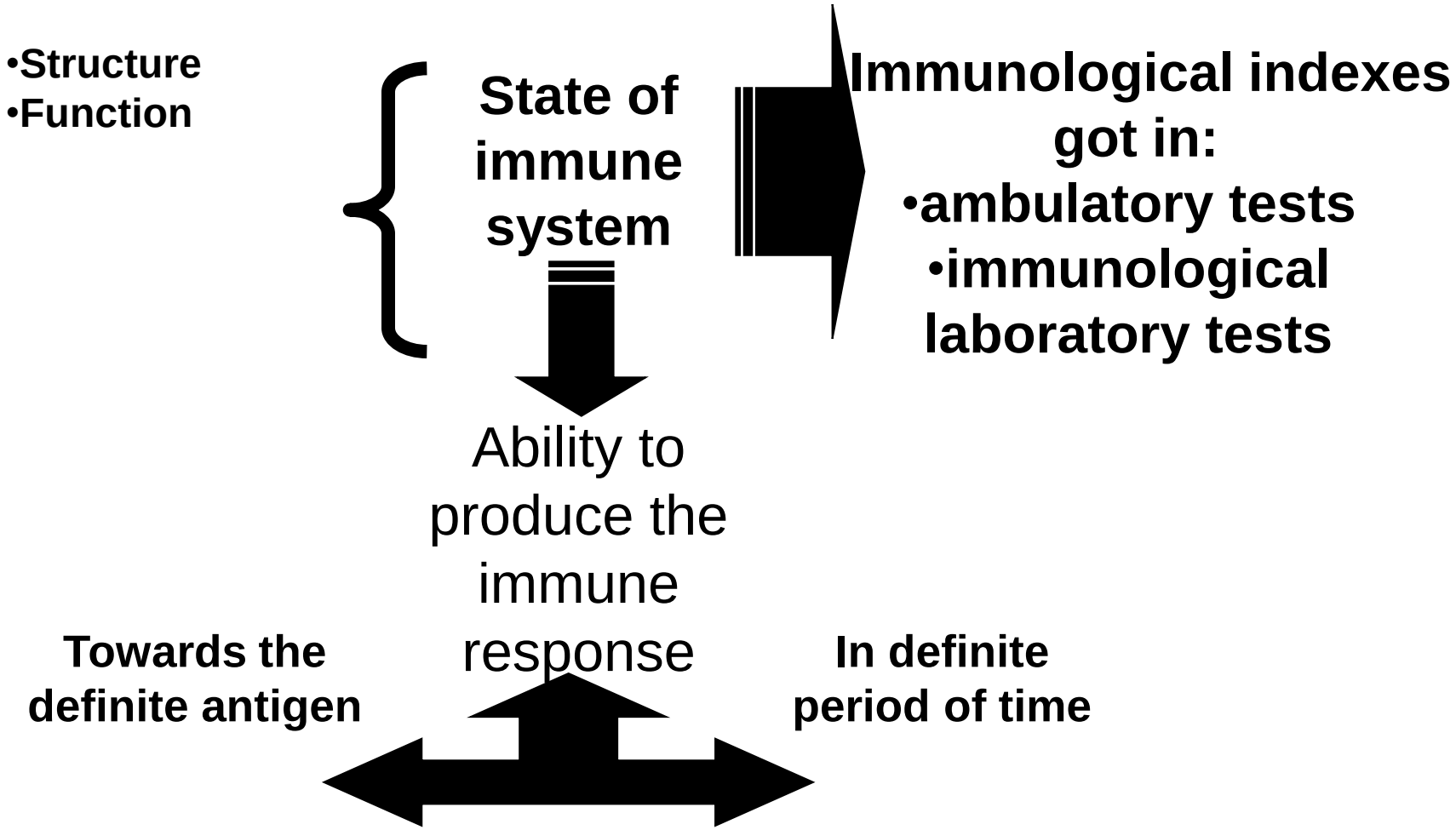
- ✓Section of Immunology that develops laboratory tests necessary for evaluation of human immune status = section of medicine that studies pathological states appeared as a result of ***human immune system functional infringements.***

«IMMUNE STATUS»(general statement)

- structure and function of immune system of the individual human organism which are defined by clinical and laboratory indexes;

- it characterizes the ability of the individual human organism to produce immune response towards the definite antigen in the definite period of time.

Scheme that describes the concept of immune status



The factors which influence the immune status

- climatological,
- geographical,
- social,
- industrial,
- ecological,
- medical.

Methods of the immune status evaluation

- the data of clinical checkup (ambulatory examination)
- indexes characterizing the state of the innate immunity
- indexes characterizing the state of humoral immunity
- indexes characterizing the state of cell-mediated immunity
- results of some additional tests

Oral rules for the evaluation of the immunograms

- ✓individuality
- ✓complexity
- ✓only significant: about 20 – 40% shifts in the value of indexes, should be taken into account
- ✓correlation between different indexes

IMMUNITY IN TRANSPLANTATION AND CANCER

MHC antigens in graft rejection

- **Class I MHC antigens** determine the graft rejection when grafted between two genetically different individuals.
- **Alien MHC antigens** cause a very strong immune response resulted in rejection.

Types of graft

- **Xenograft:** grafts between members of different species.
- **Allograft:** grafts between two members of the same species.
- **Isograft:** grafts between members of the same species who are genetically identical (identical twins).

The duration of graft survival follows the order: xeno- < allo- < iso- = auto- graft.

Humoral immunity in graft rejection

- ***Hyper-acute rejection***

  (within minutes to hours)

  occurs in blood transfusion when the recipient has preformed high titer Ab (Ig towards Ag of erythrocytes have been already synthesized):

  immediate reaction of antibodies and complement.

Cell – mediated immunity and transplantation

T-killers

Direct cytotoxic effect: thrombosis of blood vessels following by ischemia

T-effectors DTH

lymphokines synthesis

activation of phagocytes – tissue damage

Different patterns of graft rejection

Type of rejection	Time taken	Cause
Hyper-acute	Minutes-hours	Preformed anti-donor antibodies and complement.
Accelerated	Days	Reactivation of sensitized T cells: transplantation of a second graft having Ag similar with the first one.
Acute	Days - weeks	Primary activation of T cells: T lymphocytes sensitized to MHC I of allograft.
Chronic	Months - years	Causes unclear: Ab, slow cellular reactions.

Methods of suppression of the immune reactions of the graft rejection

- **Donors selection:** the most important is ***MHC*** identity with the recipient; an identical twin is the ideal donor.
- **Recipient preparation:** 1 to 5 transfusions of 100-200 ml whole blood from the donor at 1-2 week intervals improves the graft survival.
- **Immunosuppression:** most essential part of allo-transplantation (cyclosporin A).
- **Strategies for bone marrow transplantation:** the most crucial is class II MHC compatibility; the recipient must be immunosuppressed.

The reaction «graft – versus – host»

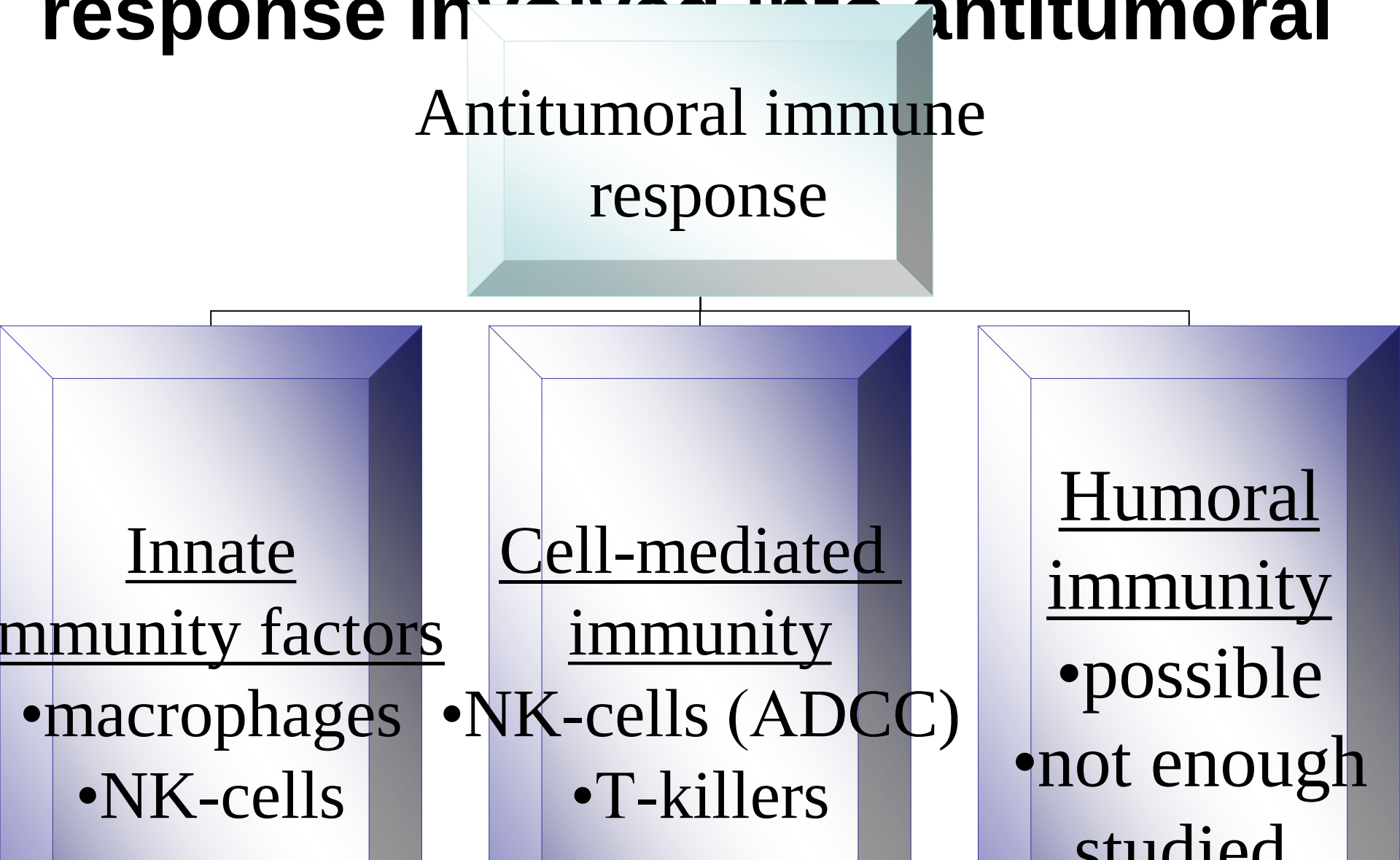
❖Graft-versus-host (GVH) reaction:

alien IC when injected into a host (recipient) recognize the allo-Ag, proliferate and cause damage to the host tissues and cells.

Tumour associated antigens

- The immune system reacts against a tumor Ag that are recognized as foreign.
- Most chemically-, physically- or virus - induced tumors have **neo-antigens**.
- In malignant cells unique **onco-fetal antigens** are expressed which not expressed by normal cells.
- Ag that cause tumor rejection - **tumor associated transplantation antigens** (**TATA**).

The general scheme of the immune response involved into antitumoral

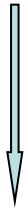


SEROLOGICAL TESTS INVOLVING COMPLEMENT (general statement)

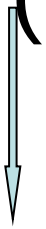
- the reactions which involve complement activation (fixation) as a result of fixing by the Ag/Ab complexes.

THE REACTION OF IMMUNE LYSIS

Ab (Ig) **+** ***Ag*** (cell) + ***C***



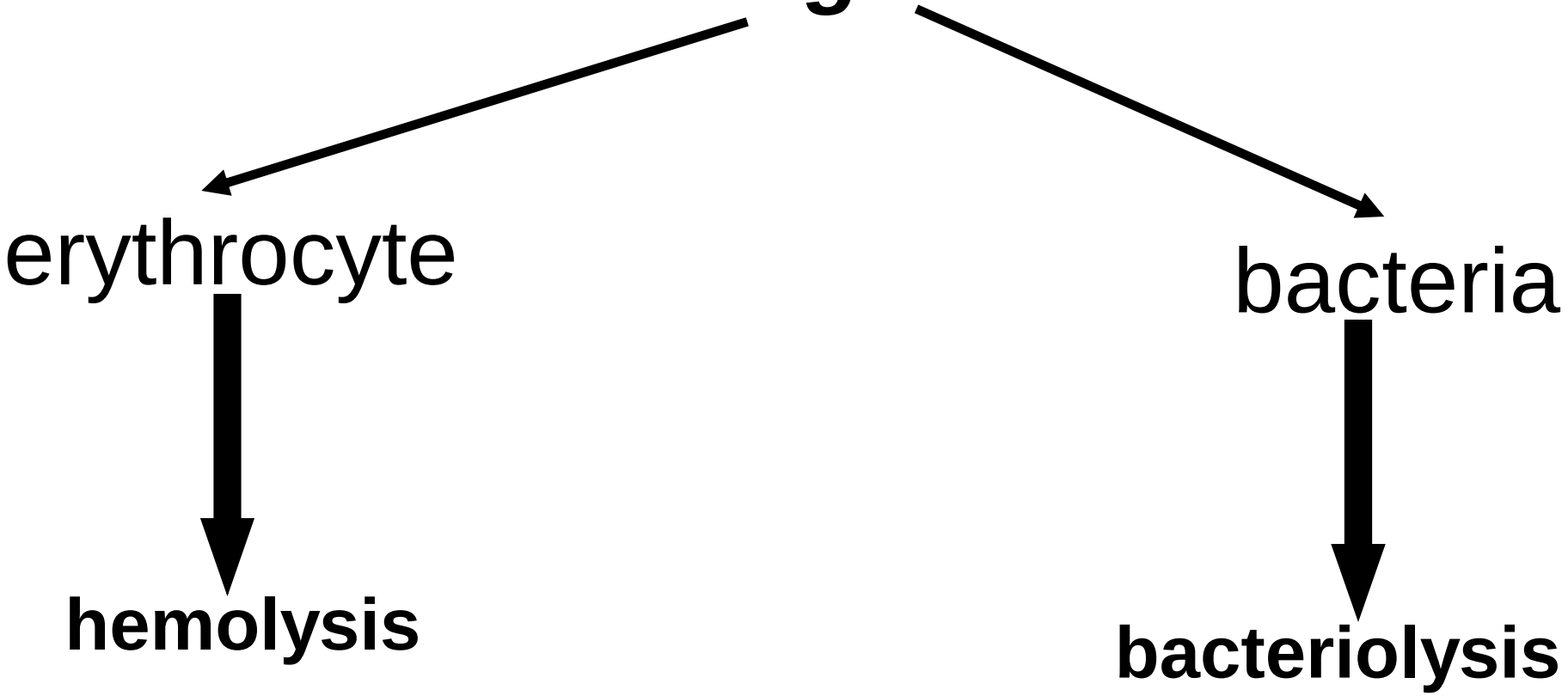
C fixation (activation)



lysis of the cell (Ag)

Types of the reaction of immune lysis

Ag



Immune adherence reaction

- ***1 STAGE:***

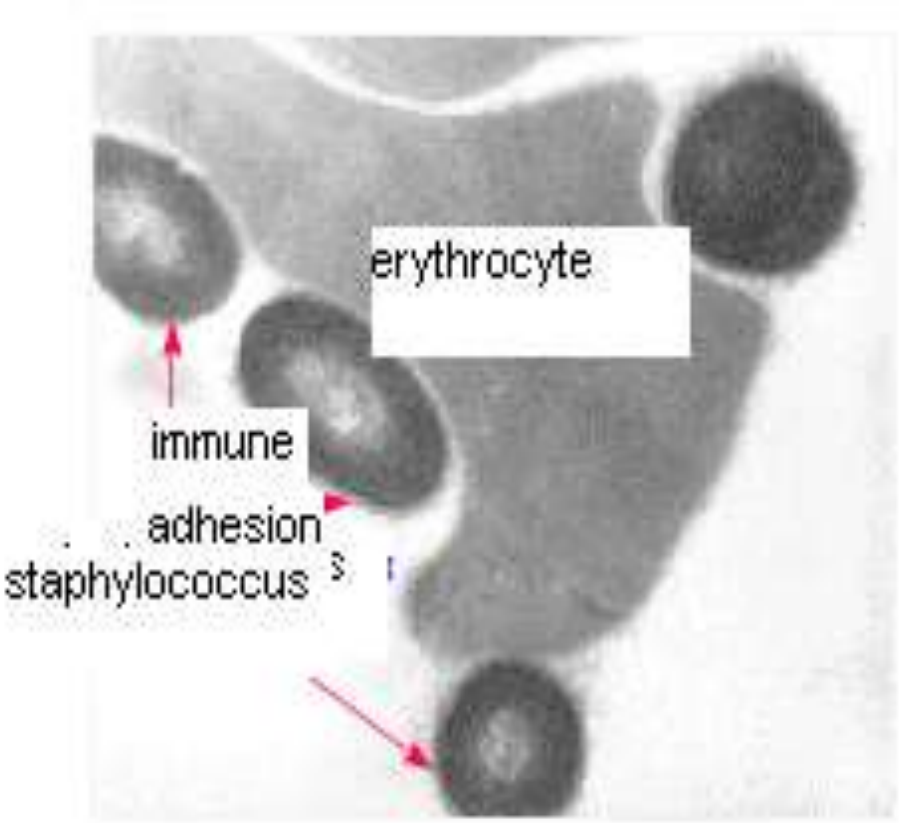
  bacterial cells + antibodies + complement → complement fixation → C_{3b} ***-binding at the surface of bacterial cells***

- ***2 STAGE:***

  + cells having receptors to C_{3b} fraction of complement (erythrocytes, trombocytes, macrophages)

adhesion of the cells to the bacteria

Immune adherence reaction

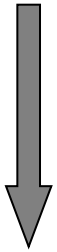


Reaction of immobilization

Ag - motile bacteria (spirochetes) +

Ab (against bacteria) + complement:

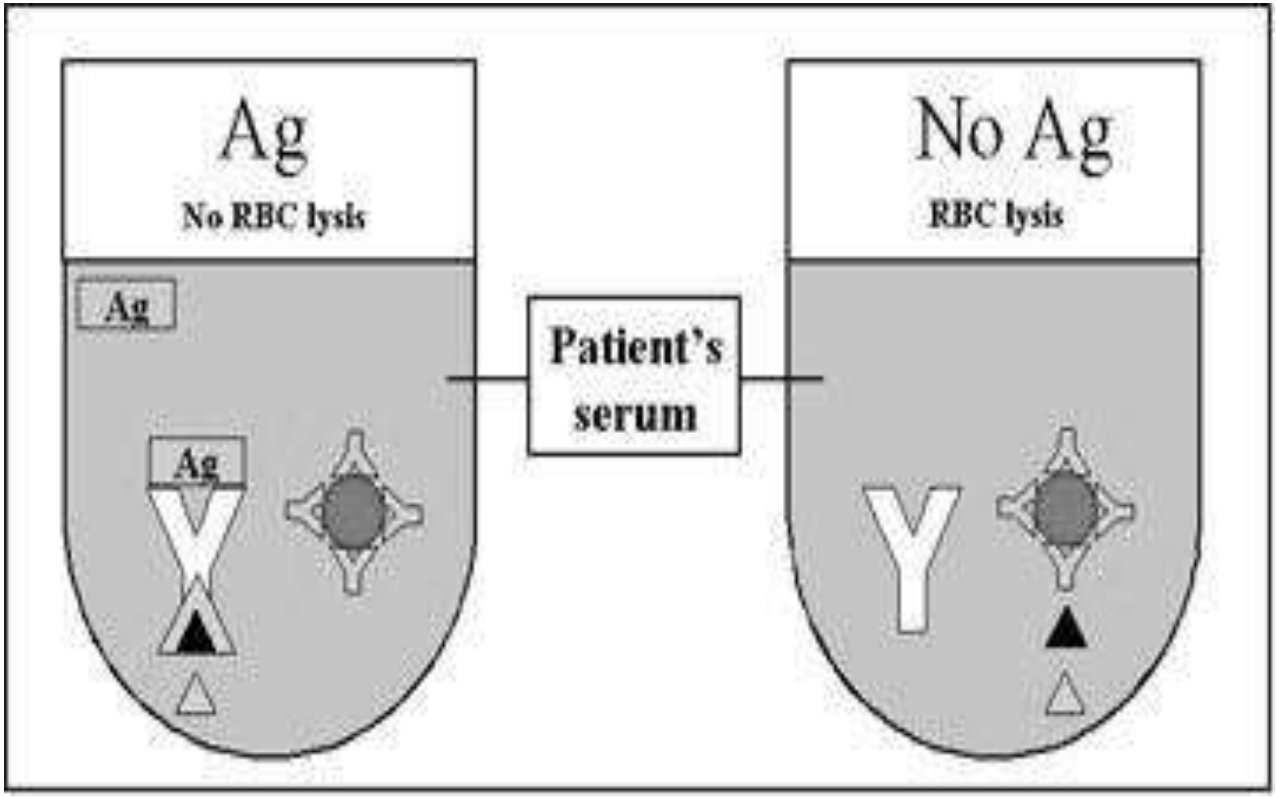
C fixation



loss of the motility by bacteria

Complement fixation reaction

- ***1 STAGE*** (general)– Ag is mixed with the test serum (Ab), incubated and Ag/Ab complexes are allowed to form.
- ***2 STAGE*** (indicative) – a standard amount of red blood cells (RBC), which have been pre-coated with anti-erythrocyte Ab is added (unbound C reacts with the RBC and causes their lysis).



- Ag/Ab complexes are formed, C will be consumed and RBC's will be not lysed
- no Ag/Ab complexes formed, C will be not consumed, it binds to the RBC and they will be lysed

IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY OF INFECTIOUS DISEASES. IMMUNITY AND AGE.

Theme № 16

IMMUNOPROPHYLAXIS: HISTORY OF DEVELOPMENT

Variolation - innoculation with pus from a patient with a mild case of smallpox has been in use for over a thousand years.

The first live vaccine was cowpox virus introduced by Edward Jenner as a vaccine for smallpox.

Edward Jenner carries out a vaccination

Immunoprophylaxis (general statements)

Immunoprophylaxis is a complex of *public health measures directed on creation of artificially acquired immunity:*

- ✓ ***active specific immune response***
  - – by vaccination

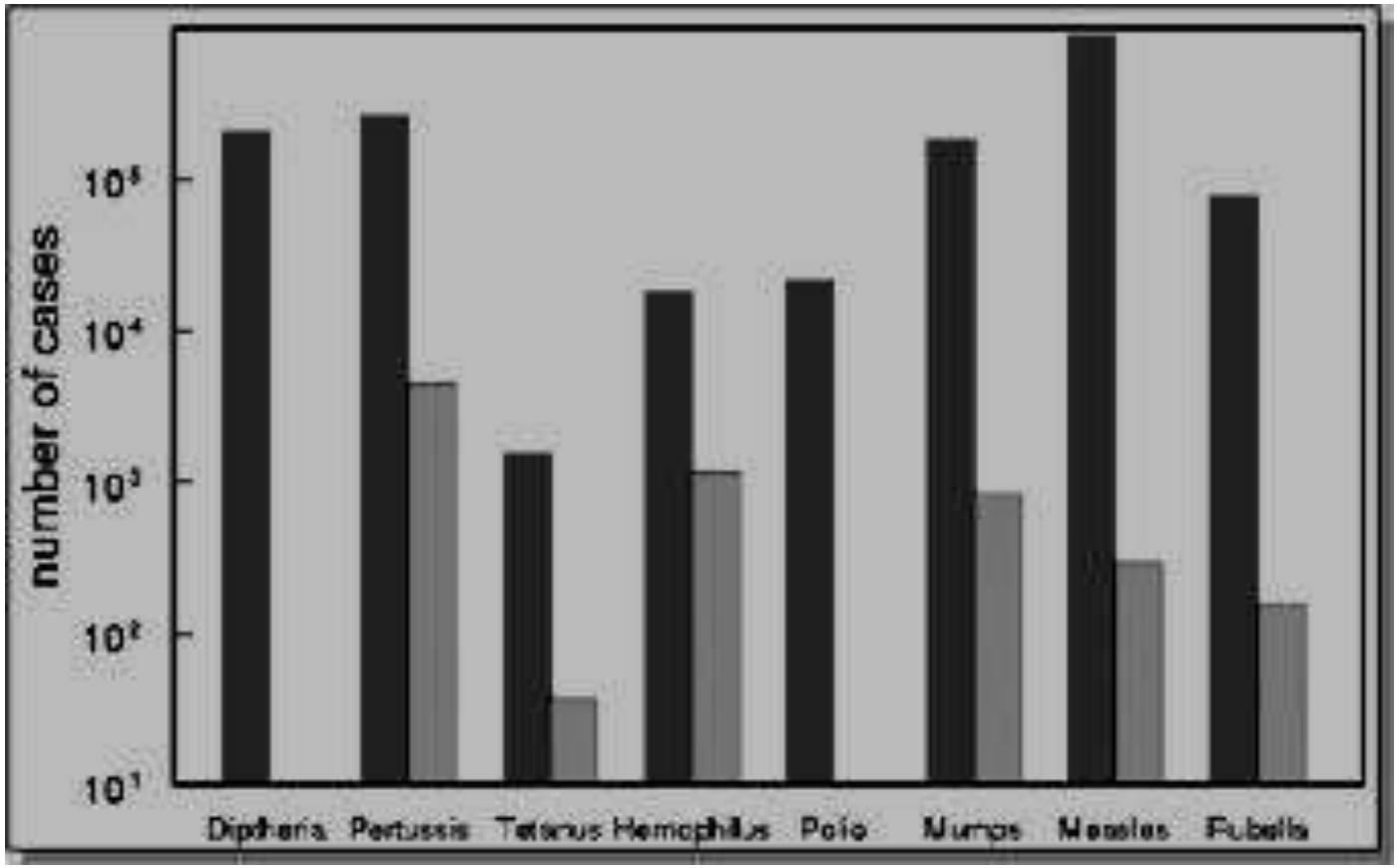
  or

- ✓ ***passive specific immunity*** –
  - – by the injection with therapeutic sera or immunoglobulins

↓

The aim of immunoprophylaxis is to provide specific protection of the human organism against infections.

Pre and post vaccine incidence of common infectious diseases



Classification of vaccines

- Live vaccines – attenuated live microorganisms.
- Killed (inactivated) - dead pathogens.
- Chemical (fractionated) - microbial components:
  - componential or subcellular (bacterial) vaccines
  - subunit or subvirion (viral) vaccines
- Molecular vaccines (toxoids) – secreted toxins which have been detoxified.
- New generation of vaccine:
  - synthetic
  - gene-spliced (recombinant), etc.

Live vaccines (attenuated)

The way of production:

- screening of stable spontaneous or induced mutants which possess strongly reduced pathogenicity and high pronounced immunogenicity **(*vaccine strain*).**

The examples of live vaccines:

- Live vaccines are used against a number of **viral** infections: poliovaccine, MMR – vaccine against measles, mumps, rubella; vaccines against chicken pox, hepatitis A, yellow fever, etc.
- The only example of **live bacterial vaccine** is vaccine against tuberculosis - **BCG**.

Live Attenuated Vaccines

- polio*
  - not used in std. schedule
- measles, mumps & rubella
- Varicella zoster
  - children with no history of chicken pox
- hepatitis A
  - not required in SC
- yellow fever
  - Military and travelers
- tuberculosis
  - not used in this country

Live vaccines

General characteristics:

- artificially acquired immunity is practically adequate to postinfectious one,
- live vaccine induces *vaccine process* – propagation of the vaccine strain in the human organism that causes self-limiting non-clinical infection following by activation of immune cells and appearance of specific memory cells,

- frequently only single introduction of vaccine is enough to induce long-lived appropriate immunity (most of vaccine strains are good immunogens),
- the protective immunity conferred by a vaccine may be lifelong (measles, tuberculosis, etc.) or may last only six months (cholera),
- live vaccines are very dangerous for immunocompromised patients: carry a serious risk of causing overt disease,
- vaccine strains could be genetically unstable (reversion to virulence).

Killed vaccines (inactivated)

The way of production:

- Inactivation of microorganisms (results in loss of pathogenicity) by the exposure to denaturing agent: effect of the *high temperature, UV irradiation or chemicals.*
- Effect of denaturing agent shouldn't cause a damage of the antigen structure – to avoid loss of immunogenicity.
- Viral killed vaccines are vaccines against polio (Salk vaccine), rabies , etc.
- **Most bacterial vaccines** are killed organisms (typhoid, pertussis, *etc*.).

Killed Whole-Organism Vaccines

- polio
- influenza
  - elderly and at risk
- rabies
  - post exposure
- Q fever
  - population at risk
- typhoid, cholera, plague
  - epidemics and travelers
- pertussis
  - replaced by the acellular vaccine

Killed vaccines

General characteristics:

they are safer (no risk of reversion) but not so much effective then live vaccines

CHEMICAL VACCINES

The way of production:

isolation of protective antigens - bacterial cell wall components from **bacterial cells** (*componential or subcellular vaccines*) or

components of **virions** (*subunit or subvirion vaccines*)

with use of the next physicochemical methods: precipitation by alcohols and neutral salts, chromatography and ultracentrifugation.

Microbial Fragment Vaccines

- *Bordetella. Pertussis*
  - virulence factor protein
- *Haemophilus influenzae B*
  - protein conjugated polysaccharide
- *Streptococcus pneumoniae*
  - Polysaccharide mixture
- *Neisseria meningitidis*
  - polysaccharide

CHEMICAL VACCINES

General characteristics:

- ✓the safest vaccines,
- ✓their effectiveness is dependent on the preparation.

MOLECULAR VACCINES

(anatoxins or toxoids)

The way of production:

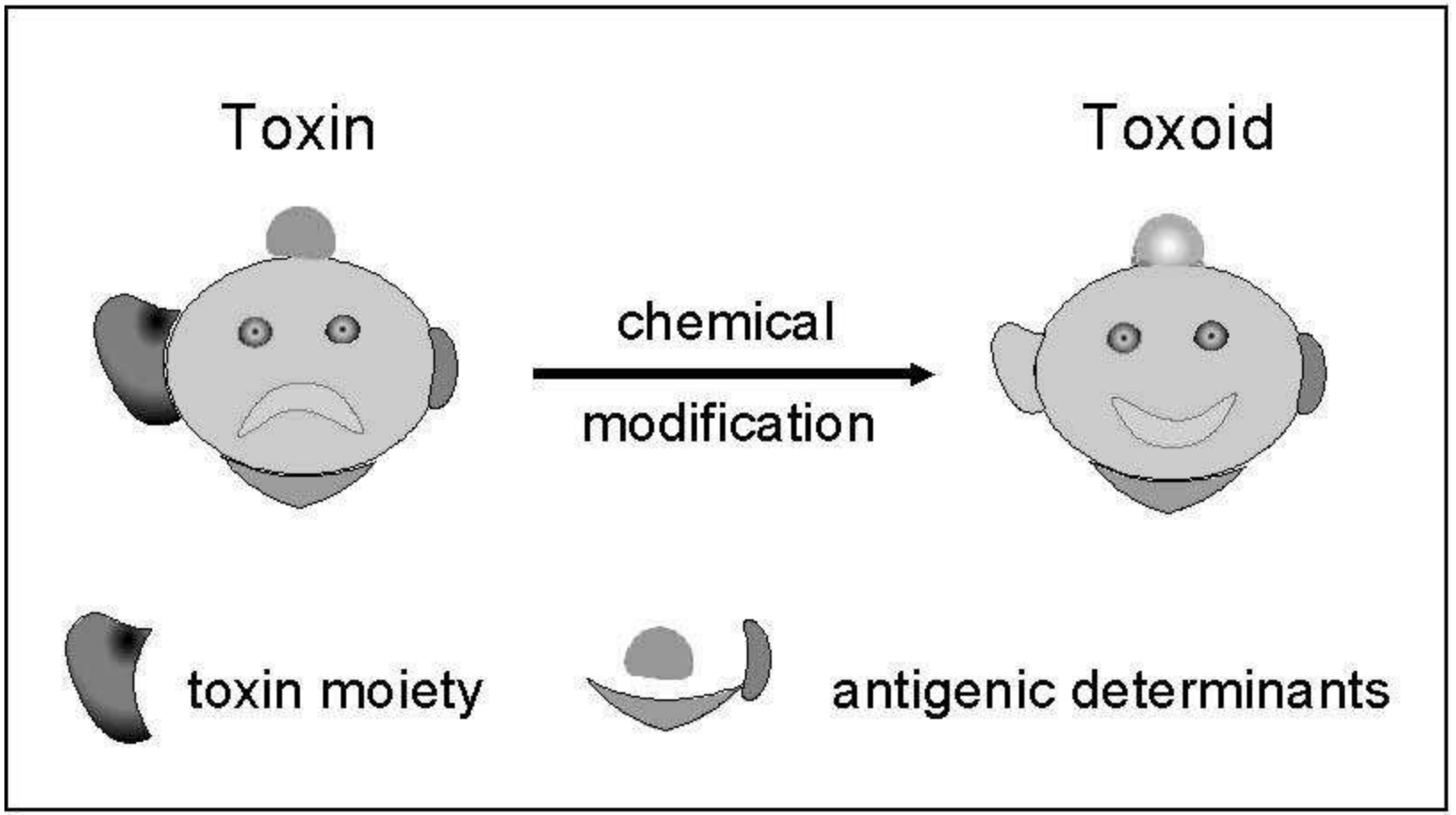
by treatment of the toxin with 0,3% formalin at 37°C during 30 days;
as a result protein toxin looses its poisonness but preserves its immunogenicity.

General characteristics:

the most effective vaccines.

Examples of toxoids: vaccines against
diphtheria, tetanus, cholera, etc.

Modification of Toxin to Toxoid



NEW GENERATION VACCINES

- gene-spliced or recombinant - some viral vaccines (hepatitis-B, rabies, *etc*.) consist of antigenic proteins cloned into a suitable vector (*e.g.*, yeast),
- synthetic – some components of vaccines (e.g. epitopes) are newly synthesized,
- immunodominant peptides (recognized by the MHC molecules) - for protection against viral diseases, - are under investigation.

VACCINE PROPHYLAXIS

Planned vaccination -

The primary immunization may be given to all children:

at the age of 2 - 3 months against hepatitis B, diphtheria, pertussis, tetanus and poliomyelitis – with use of absorbed diphtheria /pertussis / tetanus vaccine (DPT) and poliovaccines;

at the age of 13 - 15 months against mumps, measles, rubella – with use of MMR vaccine.

Table 1 Schedule for Active Immunization of Normal Children*

Age / Vaccine	Months								Years		
	1	2	4	6	12	15	18	24	4-6	11-12	14-16
Hepatitis-B ¶	HeB	HeB		HeB						HeB	
Diphtheria, Tetanus, Pertussis &		DTaP	DTaP	DTaP		DTaP			DTaP	Td	
Hemophilus influenzae-b (CV)		Hib	Hib	Hib	Hib						
Poliovirus ++		IPV	IPV	IVP					IPV		
Measles, Mumps, Rubella					MMR				MMR	MMR	
Varicella $					Var						
Hepatitis A &&								HepA			

*Recommended by Advisory Committee on Immunization , American academy of Pediatrics (2000).

¶ Infants of HbS-Ag mothers receive Hb-immune Ig within 12 hours and then the standard schedule. Adolescents not immunized can begin their series on any visit.

& Acellular Pertussis is preferable but whole cell preparation is acceptable. Tetanus diphtheria (Td) adsorbed preparation is recommended at 11-12 years and every 10 years.

++ Inactivated Polio vaccines is recommended as the standard.

$ Varicella zoster recommended at 12 months for infants who have not been exposed to or are suspected not have been exposed to chicken pox.

&& HepA recommended for use in certain areas, manly in the west (not needed in SC)

Vaccination in the case of epidemiological reasons:

- in definite regions where some endemic infections could occur,
- in the case of the professional contacts with infectious agents,
- in the case of danger of epidemics or infectious disease (epidemics of flu or bite of suspicious animal).

COMPLICATIONS OF VACCINATION

- ✓ the cross-reactive antigens of vaccine strain could cause autoimmune reaction;
- ✓ some vaccines (for example, against rabies) could cause encephalitis;

- ✓ other pathological states: in the case when patient has acute cardiovascular diseases, diseases of respiratory system, kidney, pathologies of nervous system and other chronic diseases or immune deficiencies, strong allergic reaction, - the list of these diseases is specific for every vaccine;

- ✓ the danger of overload of the organism of children by alien antigens, especially during the first year when child gets 4 – 5 vaccinations; that can cause pronounced sensitization and appearance of heteroallergies.

Adverse event occurring within 48 hours DTP vaccination

Event	Frequency
Local:	
redness, swelling, pain	1 in 2-3 doses
Mild/moderate systemic:	
fever, drowsiness, fretfulness vomiting,	1 in 2-3 doses
anorexia	1 in 5-15 doses
More serious systemic:	
persistent crying, fever	1 in 100-300 doses
collapse, convulsions	1 in 1750 doses
acute encephalopathy permanent	1 in 100,000 doses
neurological deficit	1 in 300,000 doses

The scheme of vaccination

- Single introduction of the vaccines.
- Repeated introduction of the vaccines.

Repeated introduction of the vaccines

1) ***primary vaccination*** **– creates *ground-immunity* as it causes the appearance of the memory immune cells;**
2) ***consequent revaccinations:*** **the first one takes place usually after *3 – 6 months* and the next vaccinations are introduced with use of special scheme applied for the definite vaccine – provide long-term defensive immunity. In adults it is enough to use just minimal supportive – *booster* doses of the vaccine.**

Passive Immunity

Classification

- **Naturally acquired passive immunity** - transferred from mother to fetus through placenta with IgG.
- **Artificially acquired passive immunity -** artificially transferred by injection with gamma-globulins or sera from other individuals.

Cases when artificially transferred passive immunity is practiced

The next acute situations:

- infections (diphtheria, tetanus, rabies, etc.),
- poisoning (insects, reptiles, botulism),
- as a prophylactic measure (hypogammaglobulinemia).

Immunotherapy

General description:

- treatment of humans with immune preparations to induce an adequate immune response against definite pathogen or noninfectious alien antigen.

Preparations used for immunotherapy

1. vaccines (called vaccinotherapy),
2. therapeutic sera (called serotherapy),
3. immune modulators.

VACCINOTHERAPY

Killed vaccines

In chronic infectious diseases

effects

immune stimulation,

desensitization

Therapeutic sera and immunoglobulins

Presented by two main groups

- ✓Heterologous (horse mainly)
- ✓ Homologous (human)

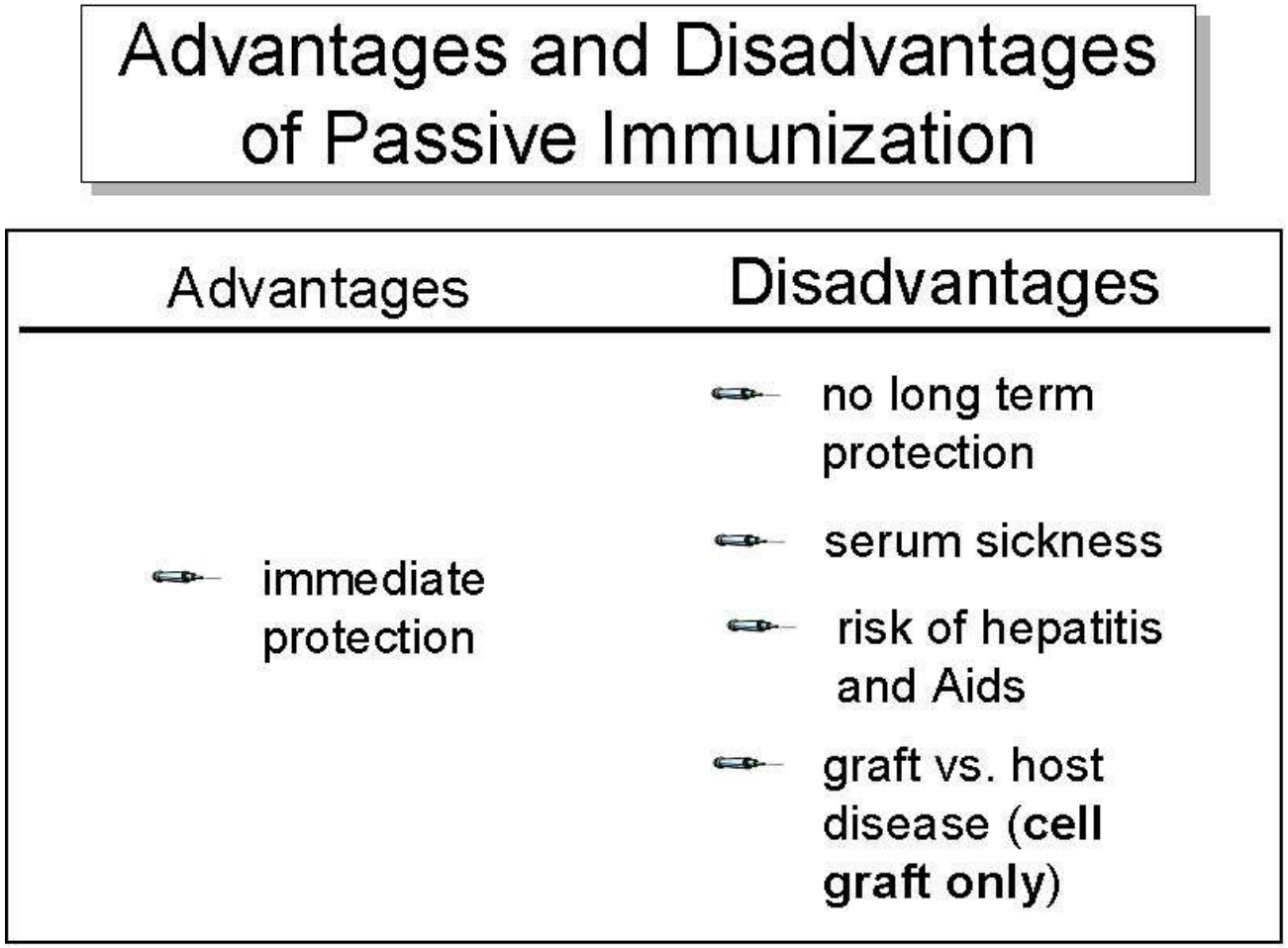
The immune preparations of human origin are preferable.

.

Complications following the introduction of alien protein (vaccine, serum)

1. strong fever
2. seroresponse
3. anaphylaxis

Advantages and Disadvantages of Passive Immunization



Immune modulators

Immune modulators

- ✓ preparations which normalize the function of immune system
- ✓ most frequently used for the stimulation of immune system

IMMUNITY AND AGE

AGEING

⇓

INVOLUTION OF THYMUS

starts already in the period of sex maturation

⇓

DECREASE

of the production of hormones in thymus

⇓

SLOWING DOWN

maturation of T-lymphocytes

⇓

DECREASE

of the number and function of T-lymphocytes

⇓

DECREASE

cell-mediated immunity (DTH)

+ moderate decrease of humoral immunity

+ autoimmune reactions

SPECIFICITY OF IMMUNITY IN NEWBORNS

Immunity of newborn

- ***1. Complement system***

- C1-C4 concentration is ≈ 2 times lower then in adult
- weak regulation

especially – of the alternative route

Low opsonising activity of blood

2. Phagocytosis

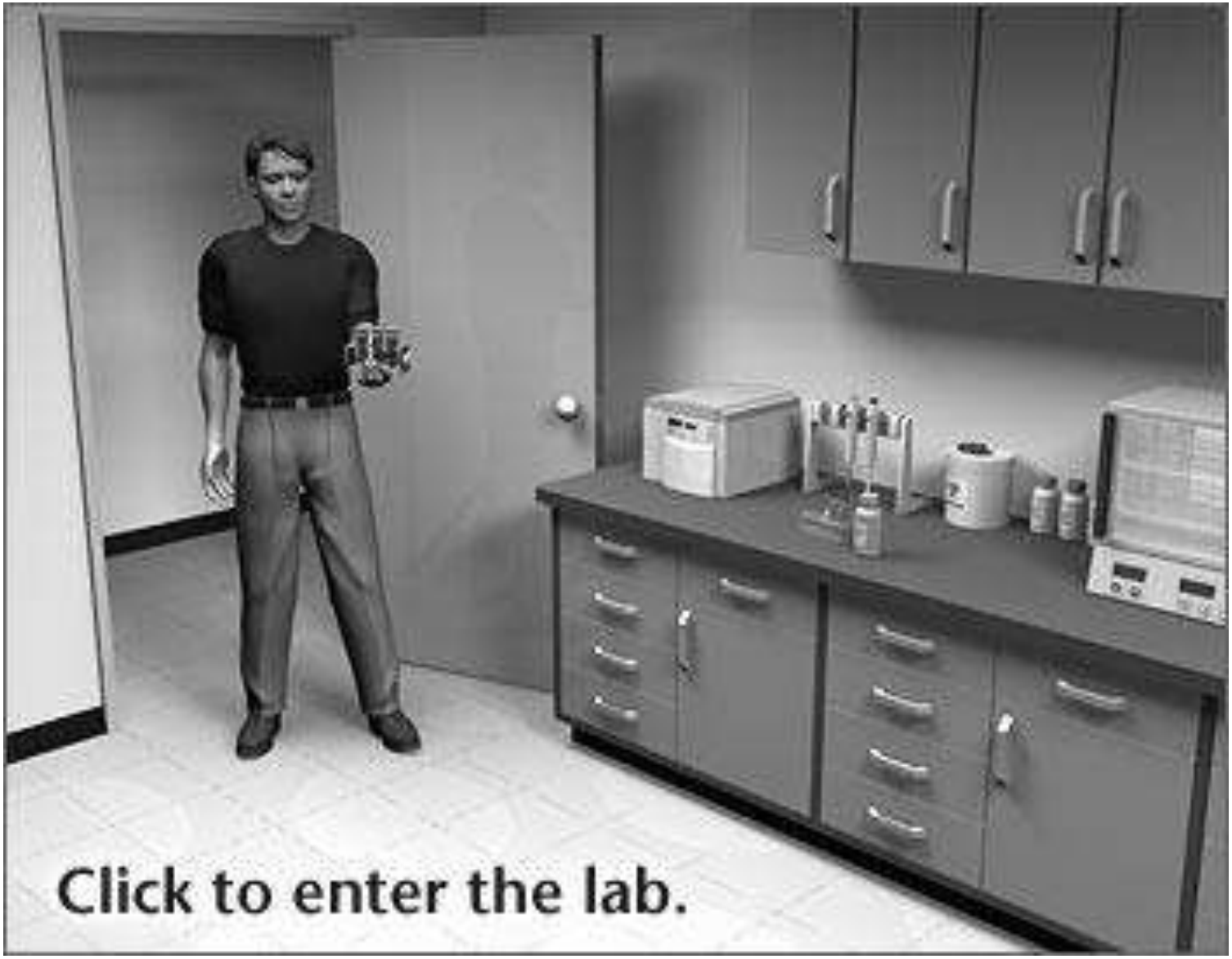
- Frequently is non-completed
- Weak reaction of chemotaxis of phagocytes

3. Immunocompetent cells (ICC)

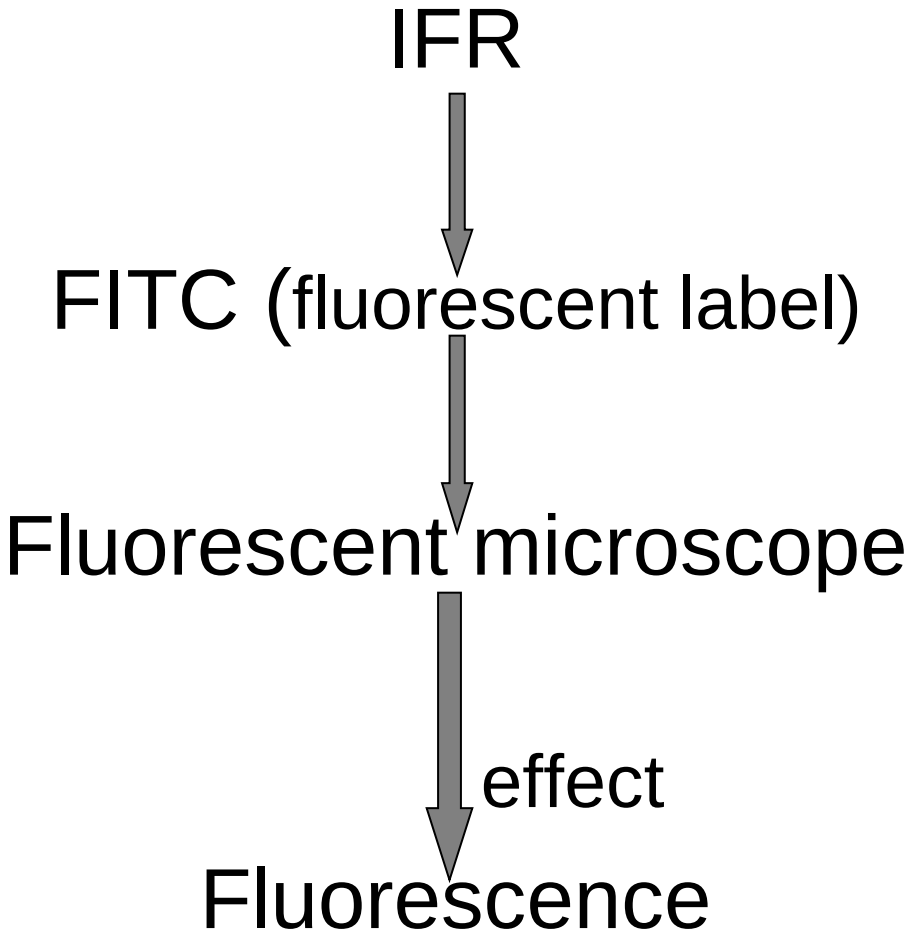
- Decreased reaction of blast-transformation of lymphocytes
- Decreased activity of cytotoxic lymphocytes (CTL) and NK-cells
- Skin tests of DTH are negative

4. **Decreased production of cytokines and interferones**

Serological tests using labelled antibodies or antigens (general characteristics)


Click to enter the lab.

Immune fluorescence reaction



Ab labeled with a fluorescent molecule is used to detect the presence of an Ag by the fluorescence emitted by the Ab bound to Ag.

Reaction of immune fluorescence (IFR, Coons' technique)

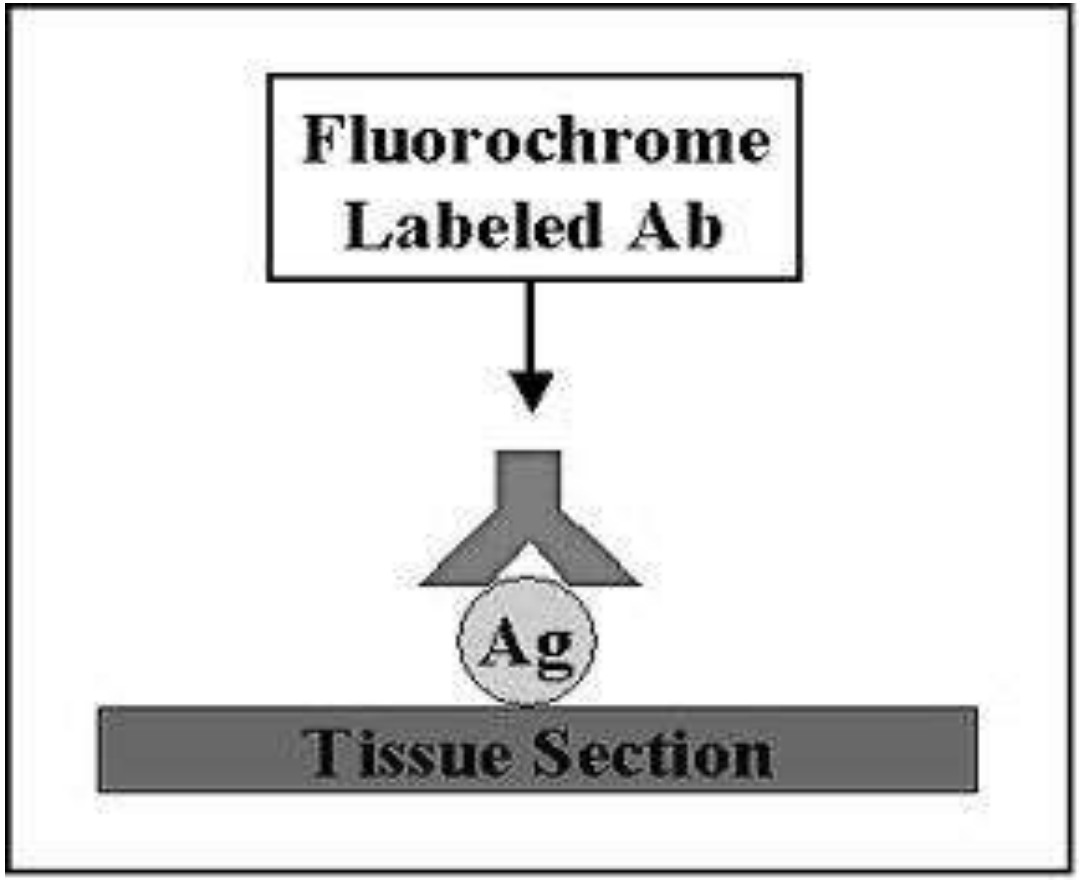
- ✓ DIRECT REACTION:

  The smear containing bacteria+ serum labeled by FITC = fluorescence visible as a green border around bacterial cells.

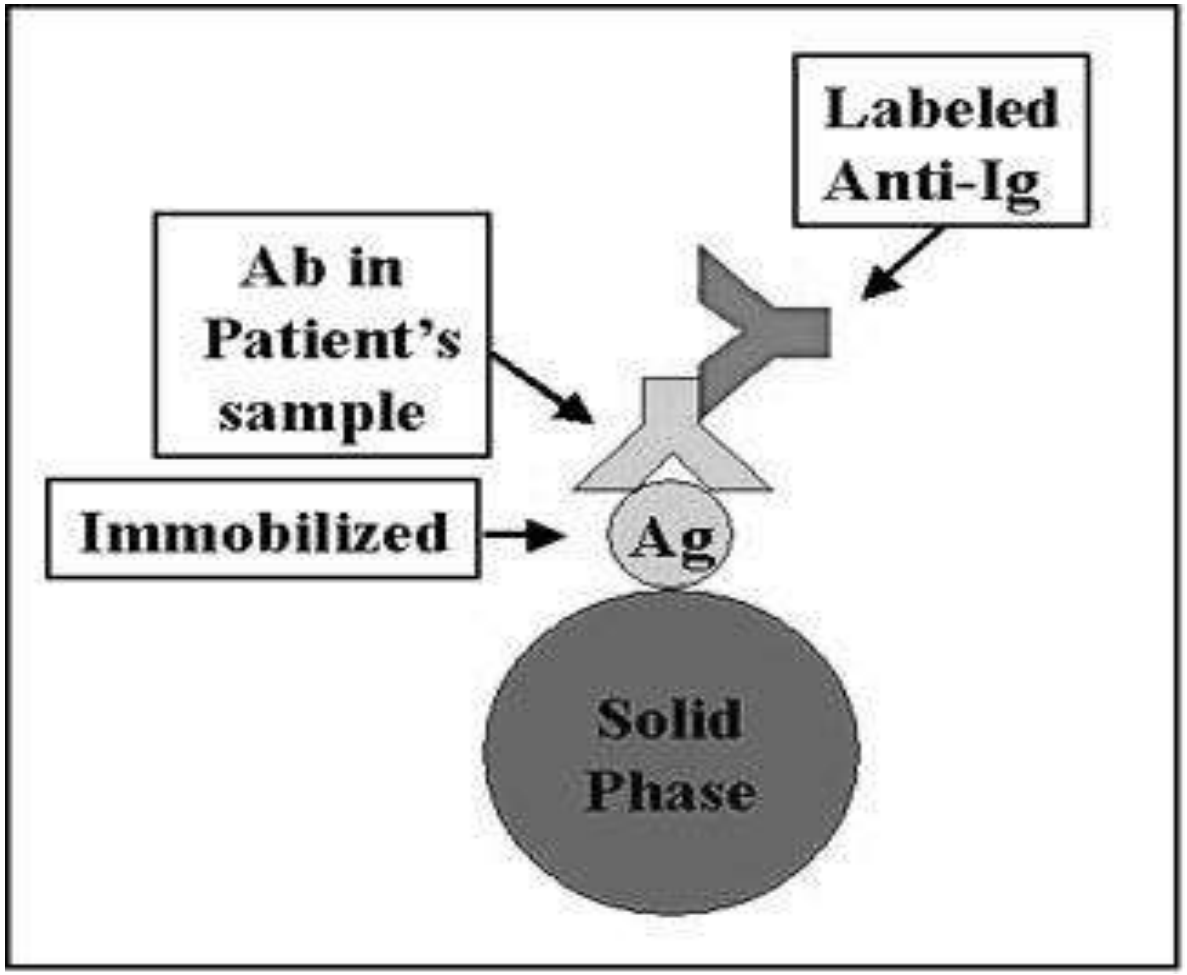
- ✓ INDIRECT REACTION:

1) the smear + diagnostic serum (usually rabbit serum)
2) wash to remove unbound antibodies,
3) +antihuman antiglobulin serum labeled by FITC = fluorescence visible as a green border around bacterial cells.

Direct IFR



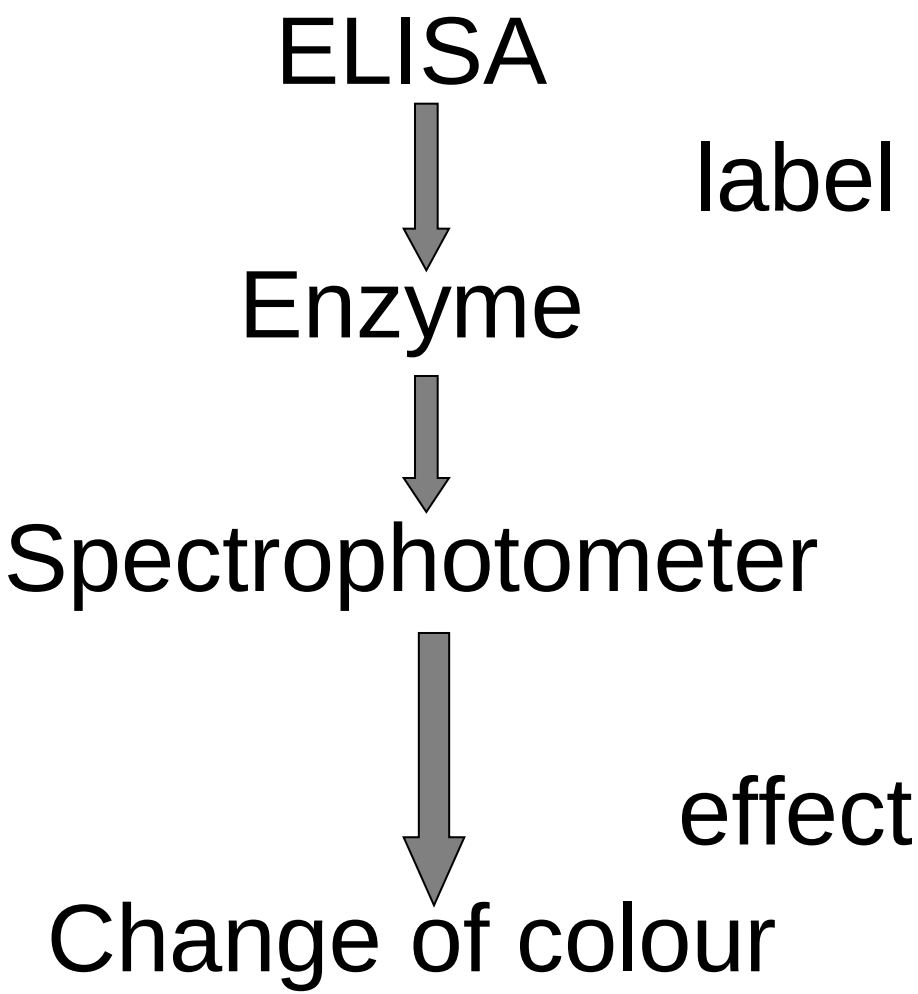
Indirect IFR



ELISA

Enzyme Linked Immunosorbent Assays is based on the measurement of an enzymatic reaction associated with Ab-Ag complexes. Enzyme may be linked to either the Ag or the Ab.

Enzyme-linked immunosorbent assay



Enzyme-linked immunosorbent assay for revealing of antibodies (stages)

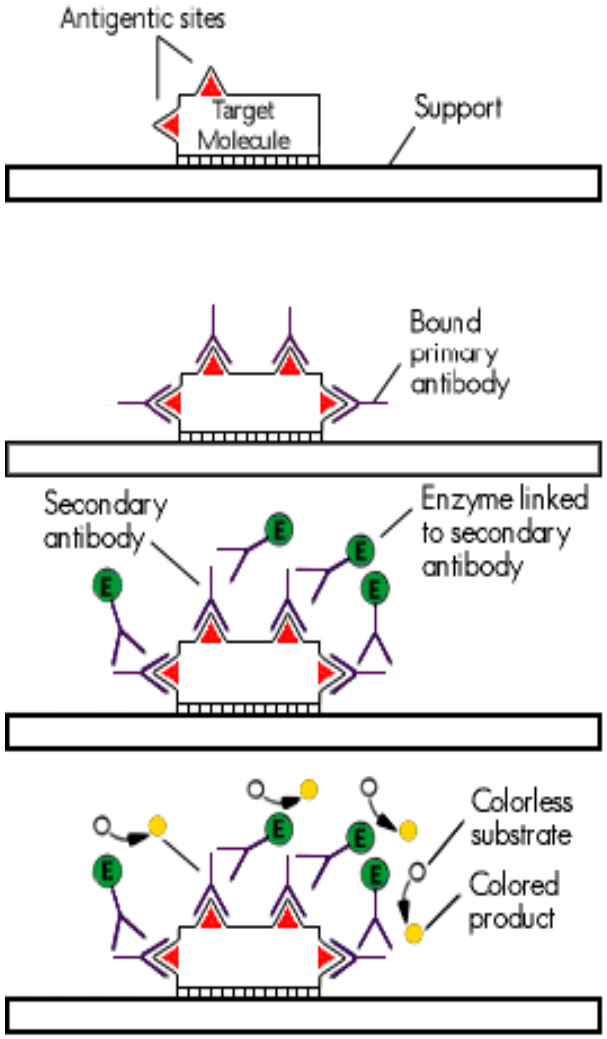
1) an antigen is attached to a plastic surface of the reading plate + ***serum of the patient***,
2) washing of the plates to remove unbound antibodies,
3) + antihuman antiglobulin serum labeled by enzyme (peroxidase or alkaline phosphatase),
4) washing of the plates to remove unbound labeled antibodies,
5) + substrate + chromogenic substance

   (colour - changing product) = change of the colour detected with use of spectrophotometer

- ***When an antigen is to be identified*** the next components have to be absorbed in the wells: the antigen +specific primary antibodies + secondary antibodies labeled by the enzyme (Ab-enzyme conjugate)+ substrate +chromogenic substance.

- ELISA could be applied for the diagnostics of the infections as well as for identification of hormones, enzymes, medical preparations and other biologically active substances which are present in the tested material in minor concentrations – ***10^{-10} –10^{-12} g/l.***

ELISA: revealing of antigen



A Bind sample to support

B Add primary antibody; wash

C Add secondary antibody-enzyme conjugate; wash

D Add substrate and chromogenic substance

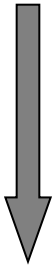
E Measurement of coloured substance with use of spectrophotometer

Radioimmunoassays

RIA - assays which are based on the measurement of radioactivity associated with Ag-Ab complexes.

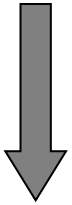
Radioimmunoassay

RIA

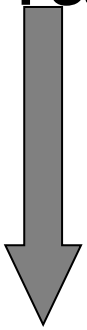


label

Radionuclides



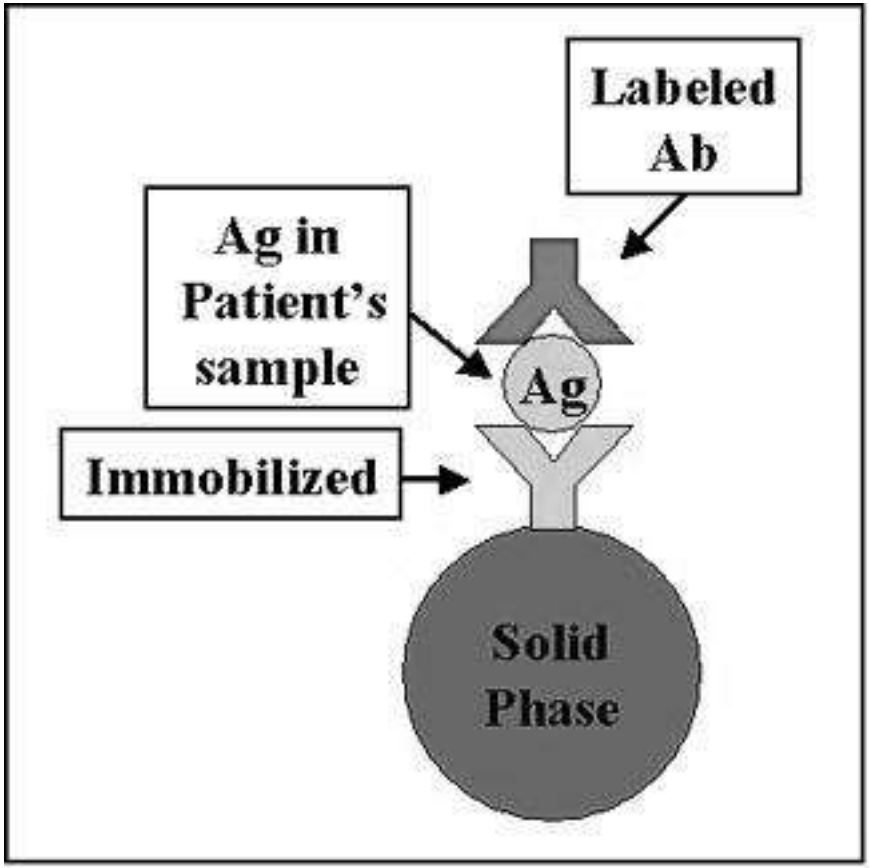
Counter of radioactivity

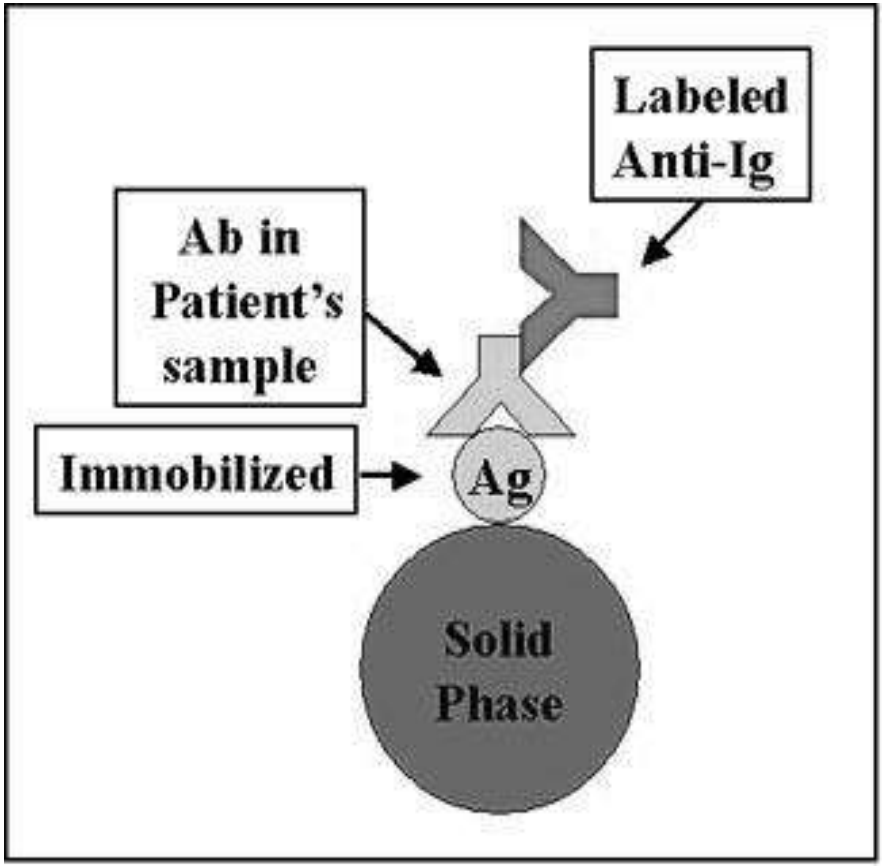


effect

Increase of radioactivity

RIA


Labeled
Ab
Ag in
Patient's
sample
Ag
Immobilized
Solid
Phase


Labeled
Anti-Ig
Ab in
Patient's
sample
Immobilized
Ag
Solid
Phase