Specific immune response

Biochemistry II
Lecture 13

2008 (J.S.)

Body's defence against microorganisms (bacteria, viruses, fungi, cells or tissues of geneticaly distinct systems:

Non-specific mechanisms -

barriers – intact skin (stratum corneum) and mucous membranes, the rinsing effect of glands secretion, ciliary epithelial cells, acidity of stomach and vagina secretion,

natural antibacterial substances – lysozyme, basic polypeptides, interferons, chemo- and leukotactic compounds,

non-induced **phagocytosis** of monocytes, tissue macrophages (histiocytes, Kupffer cells, alveolar macrophages in the lung, microglial cells), and polymorphonuclear leukocytes.

Specific mechanisms – through the mediation of the immune system – acquired **humoral immunity** and **cell-mediated immunity**.

Basal terms

Immunity is the body's ability to react on the presence of foreign protein or heteropolysaccharide (an antigen) with useful immune response as to eliminate antigens (microorganisms, transplants, tumour cells) in order to retain the molecular integrity and individuality of its own.

Immune response – the complex of reactions mediated through lymphoreticular system that follow an invasion of the foreign antigen into the body.

Lymphoreticular system (lymphoid organs or tissues) -

central, primary - thymus and equivalents of bursa of Fabricius (present in birds),

peripheral, autonomic - spleen, lymphatic nodes, bone marrow, tonsils, Peyer's patches (plaques, small intestine), T and B lymphocytes.

Antigens

Immunogens or **complete antigens**
are mostly macromolecular substances, which after they invade the body, are **recognized as foreign compounds** by immunocompetent cells, and which **initiate a specific immune response**, production of antibodies.

Haptens are small organic molecules (such as short peptides, certain drugs) that are recognized as foreign compounds but
don't initiate an immune response.

Haptens also can elicit antibodies, provided that they are attached to a macromolecular carrier, which can be quite neutral from the immunological point of view.
So the immunogen originates – a carrier with the haptenic determinant.

Antigens from the chemical point of view:

proteins and polypeptides,
saccharidic components of glycolipids and glycoproteins,
bacterial heteropolysaccharides, peptidoglycans and lipopolysaccharides,
some **nucleic acids** can act as immunogens, and
currently, some phospholipids are also mentioned.

The part of immunogen molecule that initiates the specific immune response (it can be very small) is called

a specific **antigenic determinant** or **epitope**.

On the surfaces of native protein molecules, two types of determinants are present – ether **sequential** (3 - 8 amino acid residues) or **conformational** (up to 20 amino acid residues).

Saccharidic determinants are mostly short oligosaccharides (1 - 5 monosaccharide units) at the non-reducing end.

Lymphoid stem cells of the bone marrow

differentiate postnatally to

immature T lymphocytes

maturing in the thymus or provided by thymus hormones in the periphery

immature B lymphocytes

maturing doesn't depend on thymus

clones of immunocompetent cells

with the fixed genetic information to recognize a sole specific antigen

contact with the specific antigen causes activation of cells of the particular clone

memory T cells

blastic transformation

the clone of mature T cytotoxic cells and T helper cells

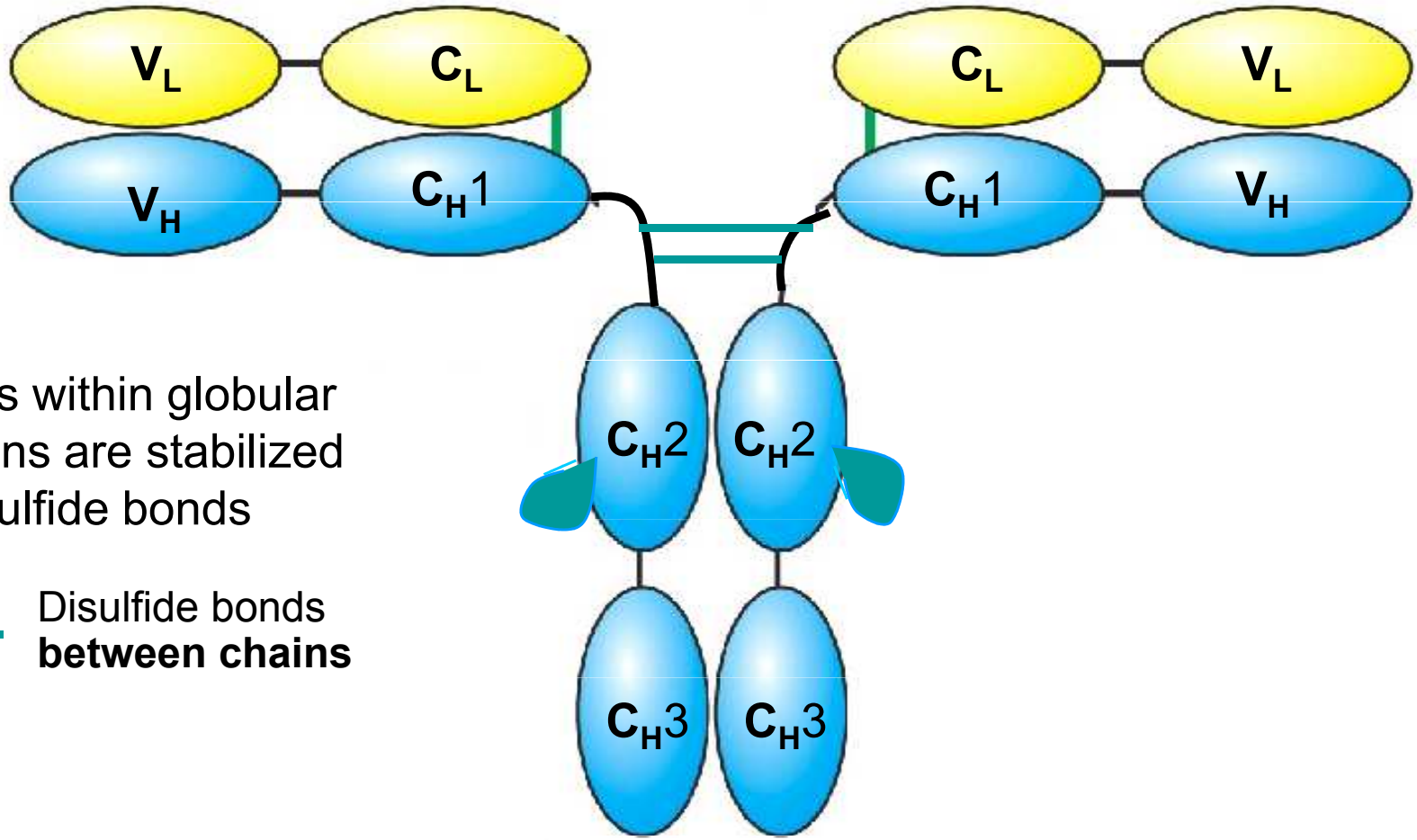
memory B cells

blastic transformation

the clone of plasmocytes producing specific antibody (immunoglobulin)

Immunoglobulin molecule (IgG)

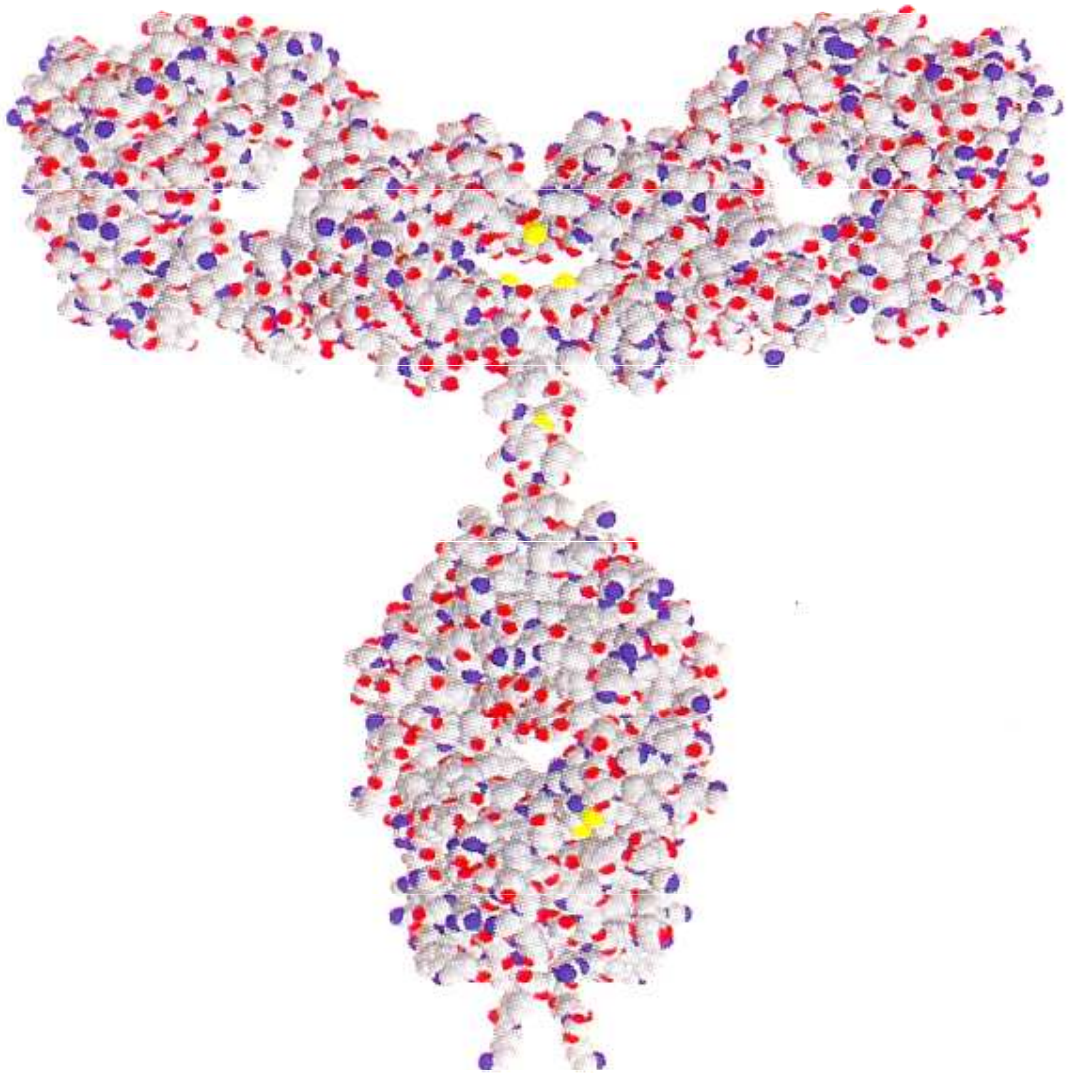
Two light chains – variable domain and constant domain
Two heavy chain – variable domain and 3(-4) constant domains



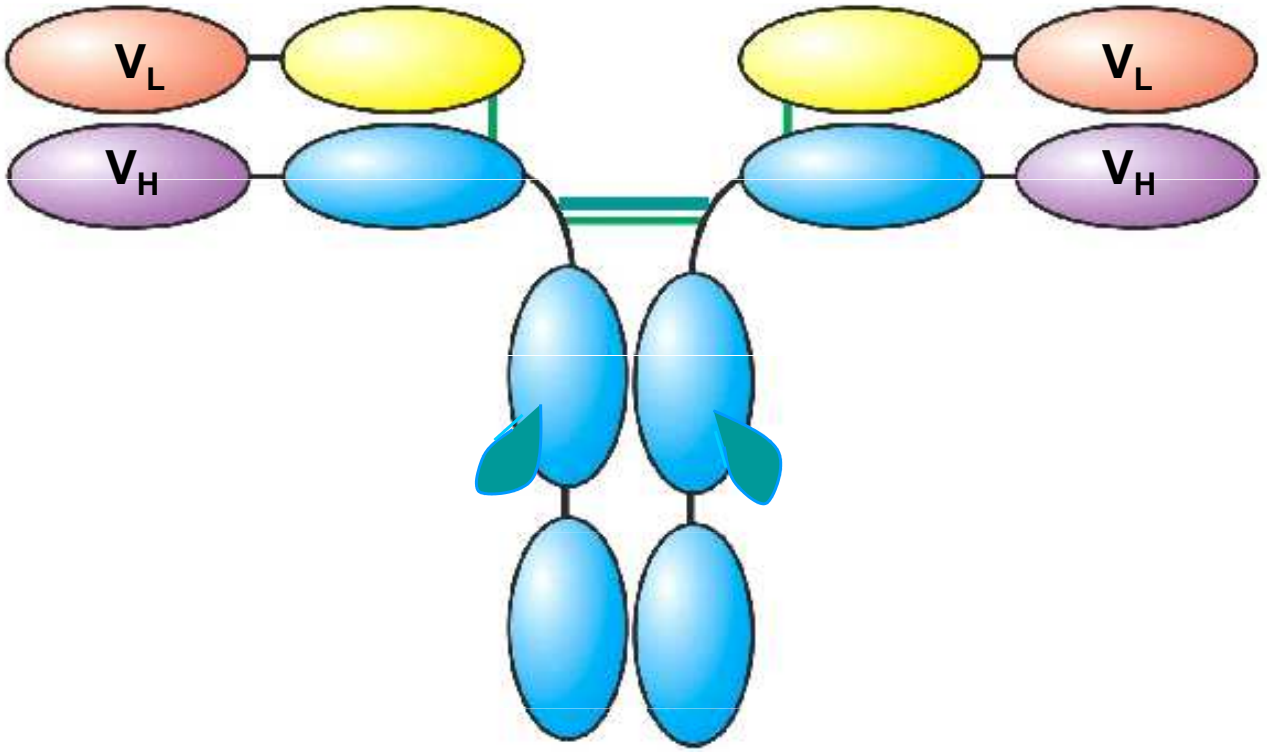
Chains within globular domains are stabilized by disulfide bonds

Disulfide bonds **between chains**

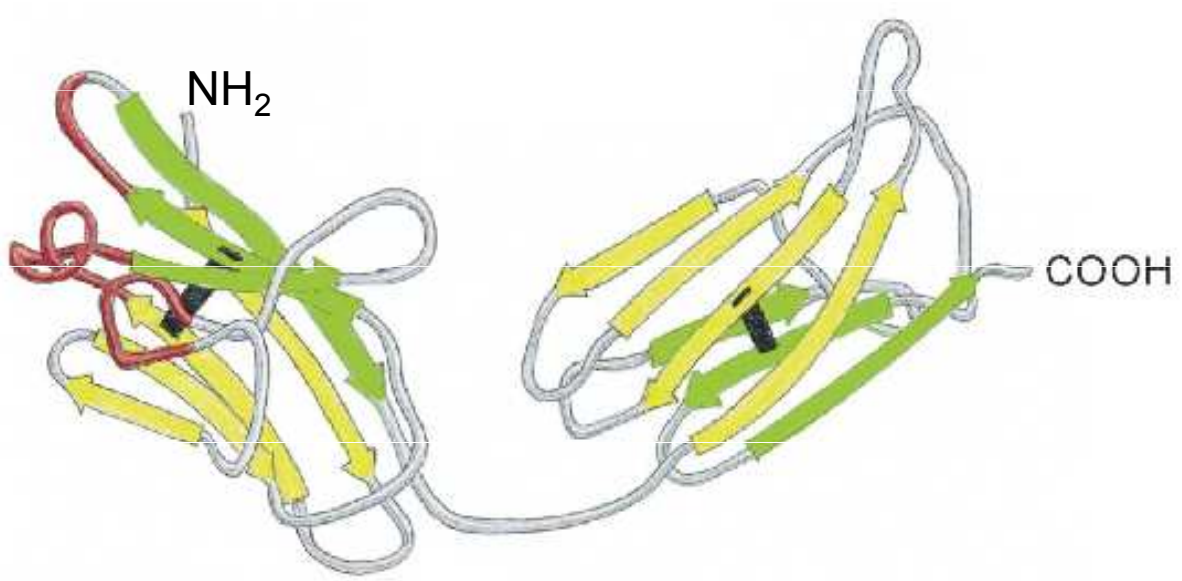
Model of immunoglobulin G



Variable domains of both heavy and light chains form at their NH_2-ends **two coincident binding sites** for the specific antigen determinant **that are quite variable from one antibody to another.**



Terciary structure of light chains



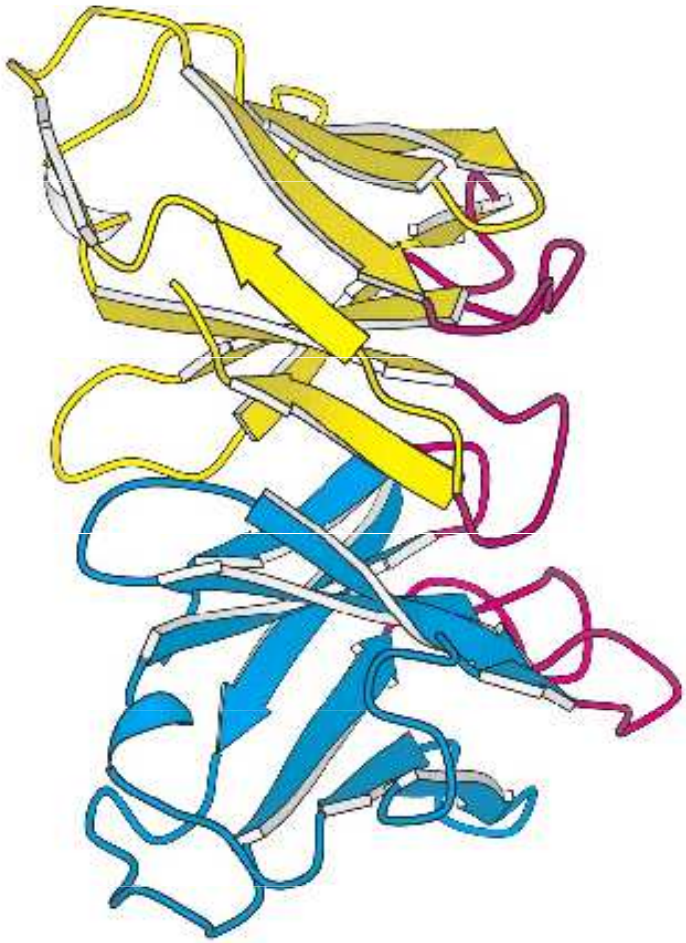
Variable domain $\mathbf{V_L}$
(3 hypervariable loops,
complementarity-determining regions)

Constant domain $\mathbf{C_L}$

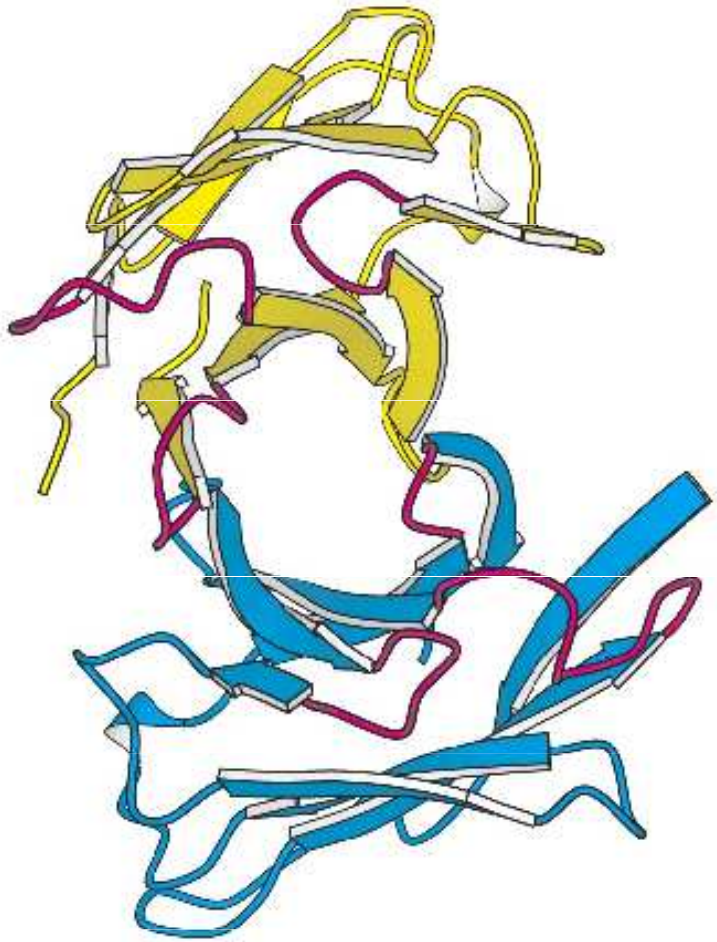
Antigen-binding site of an immunoglobulin

V_L

V_H



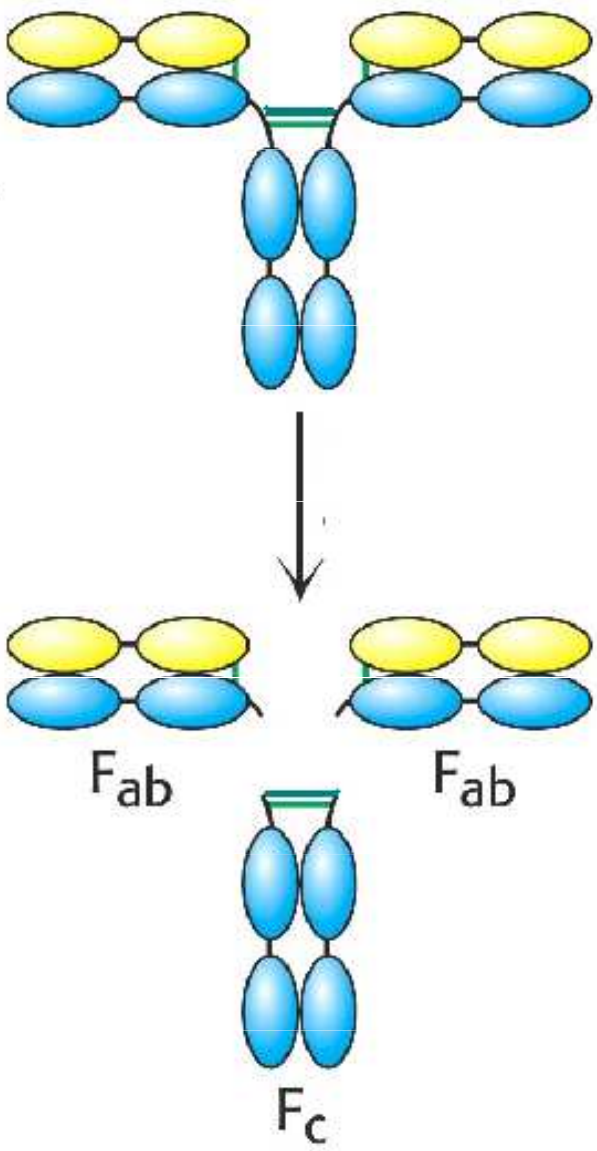
Side view



View from above in the direction of molecule axis

Treatment of intact IgG molecules with the proteinase **papain** results in the formation of three large fragments: **two F_{ab} fragments** („antigen binding“, monovalent) and **F_c fragment** ("crystallisable", it can be easily isolated in the crystalline form).

Treatment with proteinase **pepsin** results in the formation of divalent **$(F_{ab})_2$ fragment** and **F_c fragment**.



Functions of immunoglobulin domains

Variable domains V_L a V_H are responsible for the **distinctive function** of immunoglobulins, forming together a binding site for a specific antigenic determinant. Specifity of binding sites in high, it depends on the amino acid sequence of **hypervariable loops** (complementarity-determining regions, there are *three* in V_L and *four* in V_H).

Each antigen-binding site can bind noncovalently one antigenic determinant or one hapten. The strength of this interaction is called **affinity**.

As a rule, binding sites exhibit high affinity for only a limited number of similar determinants. With decreasing strength of interactions, the number of such "cross-reacting" determinants increases. Numerous determinants are bound very weakly, however these weak interactions are not significant practically.

Constant domains mediate biological functions called **effector functions**:

Interaction of variable domains with the antigen initiate the process, the result of which is antigen elimination.

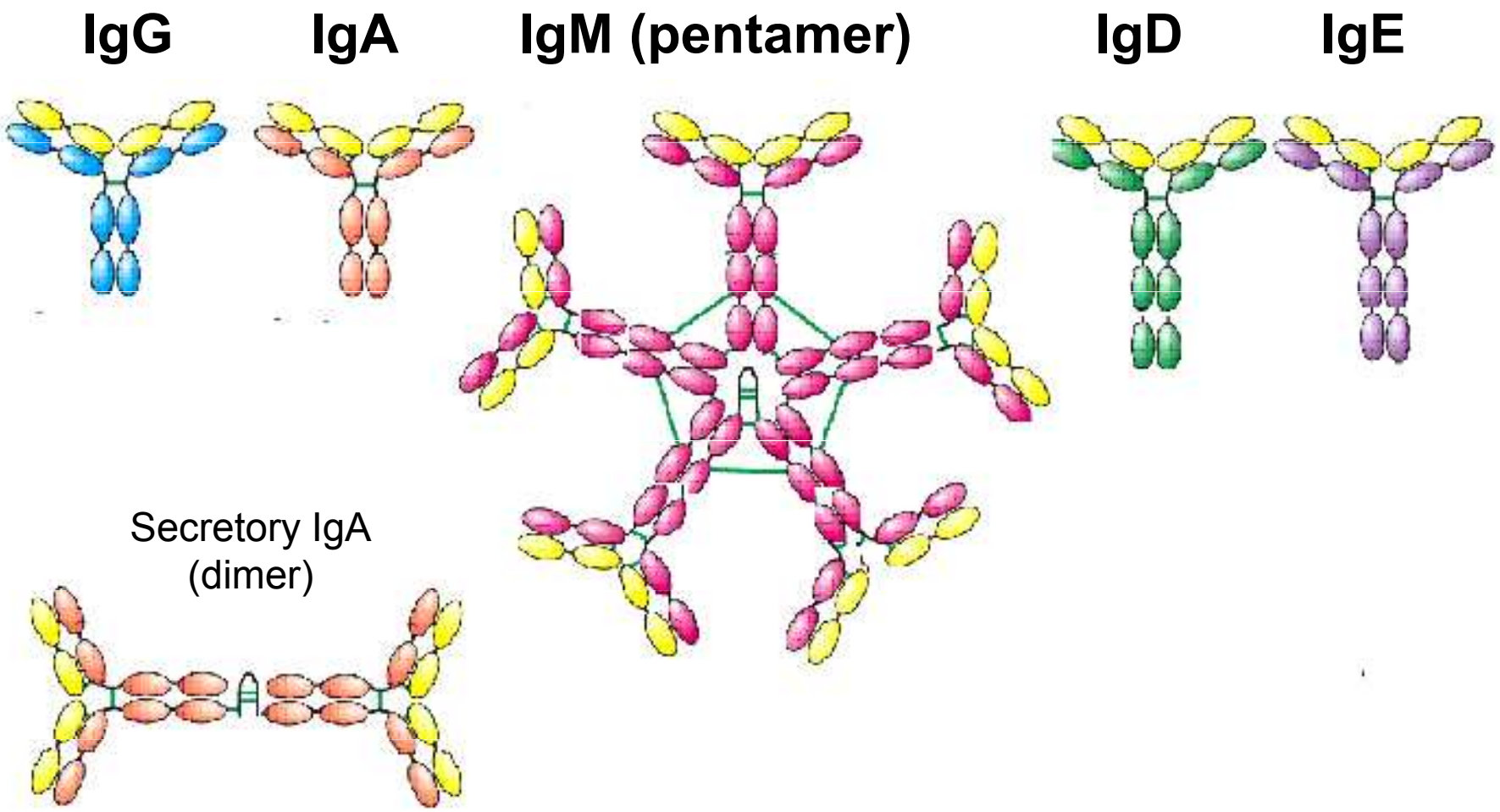
Domains **C_L a C_H1** are connected through disulfide bond. The change in conformation evoked by the interaction with antigen induces conformational changes of all remote constant domains. In the complement cascade, C_H1 domain binds the complement component $C4_b$.

The **hinge region** joins both heavy chains. In the heavy chains of IgM is the hinge substituted by special domains C_H 2.

Domains **C_H2** of immunoglobulins IgG a IgM are binding sites for the first **complement component $C1_q$** or certain immunomodulating peptides.

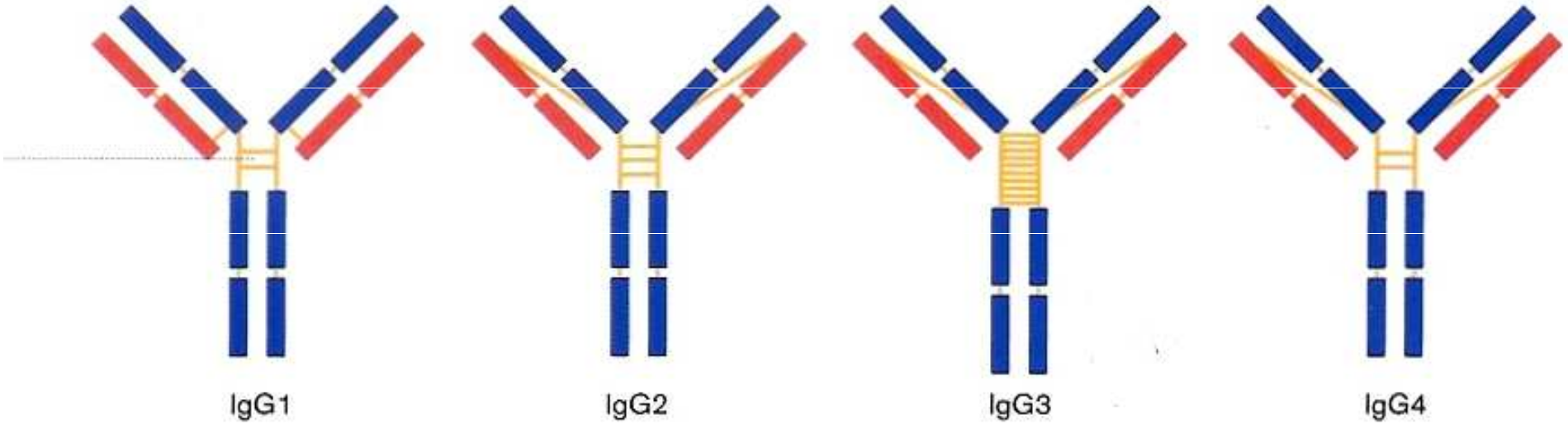
Domains **C_H3** (in IgM C_H4) enable together with domain C_H2 **cytotropic reactions – binding to F_c-receptors** of phagocytes and B or T cells, which initiates readily either phagocytosis of immunocomplexes, or formation of the complex with the cell exposing an antigen – a signal for extinguishment of the cell.

Five immunoglobulin main classes schematically



IgG - immunoglobulins class G

Subclasses (isotypes) IgG 1 – 4
differ in the numbers and positions of disulfide bridges.

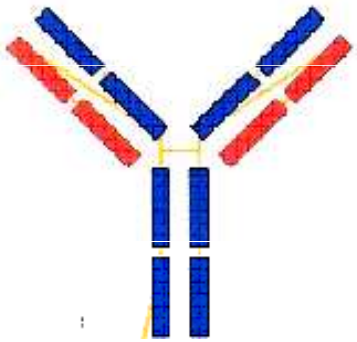


IgG 3 has up to 15 disulfide bridges between its heavy chains

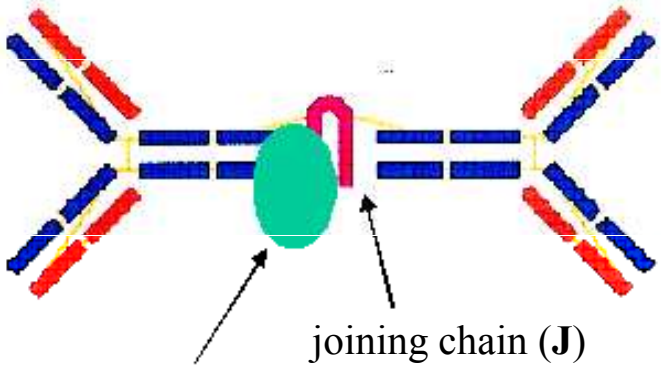
IgA - immunoglobulins class A

Subclasses (isotypes) IgA 1 and IgA 2

Serum IgA



Secretory IgA (**SIgA**, **dimer of serum IgA**)

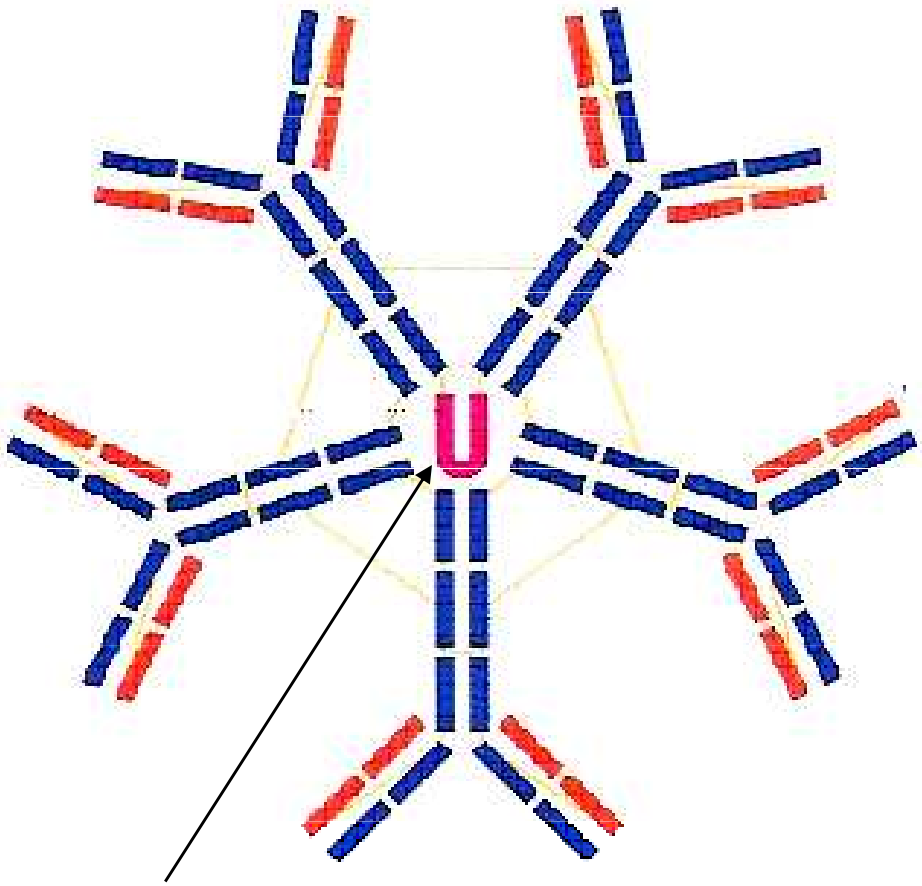


secretory component (**SC**)
*M*r 70 000

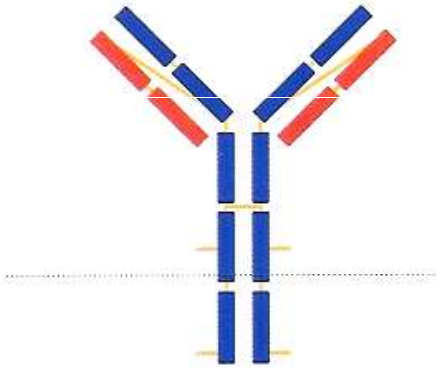
It occurs in mucous secretion, where
it takes part in reactions of local immunity.

IgM - immunoglobulins class M

Pentamers of identical basal subunits



joining chain -
a glycoprotein, M_r 15 000



Basal subunit of IgM – heavy chains comprise **four** constant domains

Classes of immunoglobulins - properties

	IgG	IgA	IgM	IgD	IgE
Heavy chains	$\gamma_1, \gamma_2, \gamma_3, \gamma_4$	α_1, α_2	μ_1, μ_2	δ	ε
Light chains	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ
Molecular formula	$\gamma_2\kappa_2$ $\gamma_2\lambda_2$	serum $\alpha_2\kappa_2$ $\alpha_2\lambda_2$ secretory $(\alpha_2\kappa_2)_2$JS $(\alpha_2\lambda_2)_2$JS	$(\mu_2\kappa_2)_5$J $(\mu_2\lambda_2)_5$J	$\delta_2\kappa_2$ $\delta_2\lambda_2$	$\varepsilon_2\kappa_2$ $\varepsilon_2\lambda_2$
Approx. M_r	150 000	180 000 - 500 000	950 000	175 000	200 000
Saccharides	3 %	8 %	10 %	12 %	12 %
Function	antibacterial and antiviral activity, complement binding	**antiviral** and antibacterial activity	**antibacterial** and antiviral activity, **complement** binding	?	reagins
Serum concentration	**~ 12** g/l	**~ 3** g/l	**~ 1,2** g/l	**< 0,1** g/l	**< 0,001** g/l

Antigen-antibody interaction

The primary event is the **formation of an antibody-antigen complex** (binding of the specific immunoglobulin to the corresponding antigen). The binding of antigens to immunoglobulins usually results in marked conformational changes.

Antigens are either soluble (colloid particles), or corpuscular (antigenic determinants on the surface of cells or other insoluble particles). Soluble Ag-Ab complexes are called **immunocomplexes**.

Two stages of the formation of immunocomplexes can be distinguished: the **binding** itself that is relatively fast (formation of non-covalent interactions, the most important of which are the hydrophobic), and the **complex transformation**, which can take longer time (the complex is stabilized through formation of more interactions).

Secondary processes associated with formation of Ag-Ab complexes

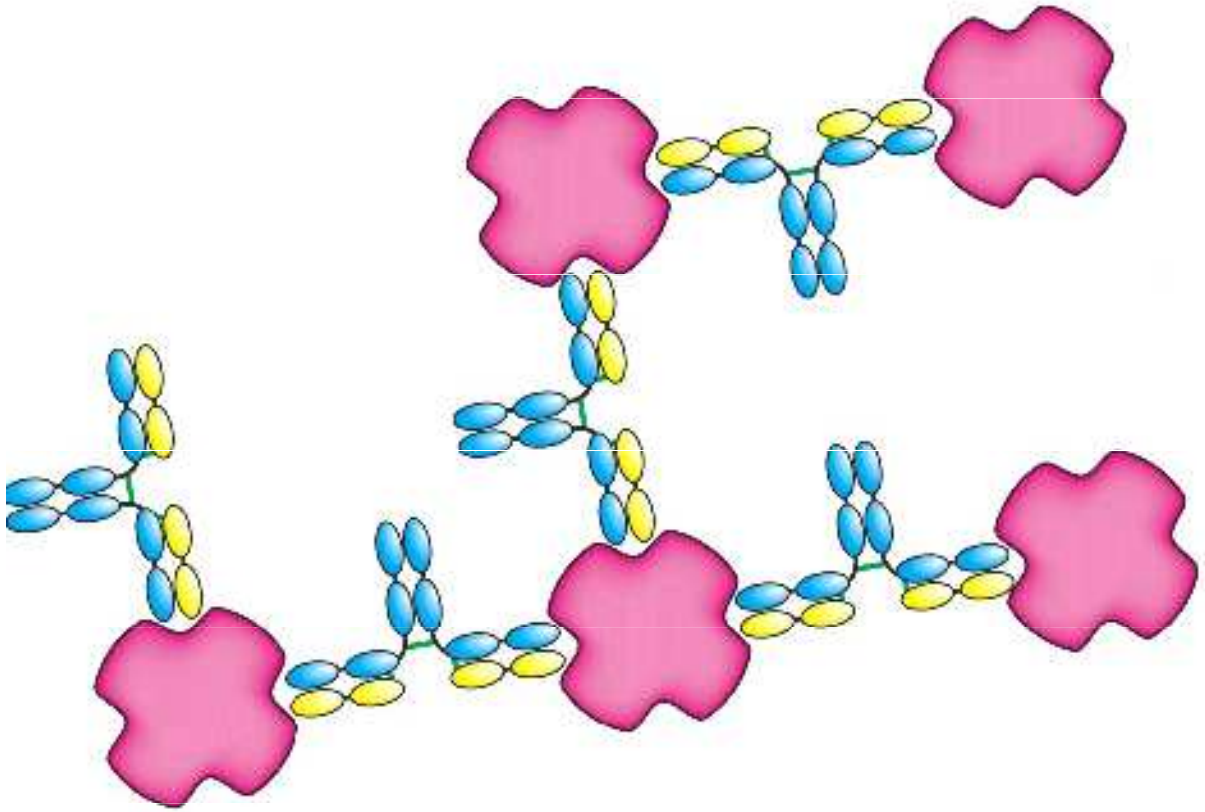
Immunoprecipitation. Immunoglobulin molecules include two antigen-binding sites, so that they can cross-link soluble multivalent antigens at certain limit concentration (and a proper concentration ratio of both). These three-dimensional networks are insoluble and visible as turbidities or precipitates. An excess of both antibody or antigen inhibits precipitate formation.

Agglutination of cells or other particles is a similar process: Immunoglobulins act as cross-links between antigenic determinants of multivalent corpuscular antigens (cells, bacteria, generally agglutinogens). Aggregates of particles (agglutinates) are easily distinguishable from sediments of particles that are not agglutinated.

Cytotropic reactions. F_c receptors bind immunocomplexes, the result may be either **phagocytosis of the immunocomplex** or (mediated by cytotoxic T cells) **extinguishment of the antigen-exposing cell**.

Triggering of the complement cascade (the classical pathway of activation of complement components) is a process that leads to the **lysis of foreign target cells**.

Antigen cross-linking in immunoprecipitates (soluble antigens) or agglutinates (corpuscular antigens)



Diversity of antibodies is generated by gene rearrangements

Antigen-binding sites of molecules responsible for humoral immunity – membrane-bound immunoglobulins of B cells (*m*Ig), receptors of T-helper cells (TCR), and antibodies produced by plasmocytes – exhibit an extreme diversity. More than **10^8 different structures** can be formed (in cells responsible for the cellular immunity even about **10^{12}** different structures).

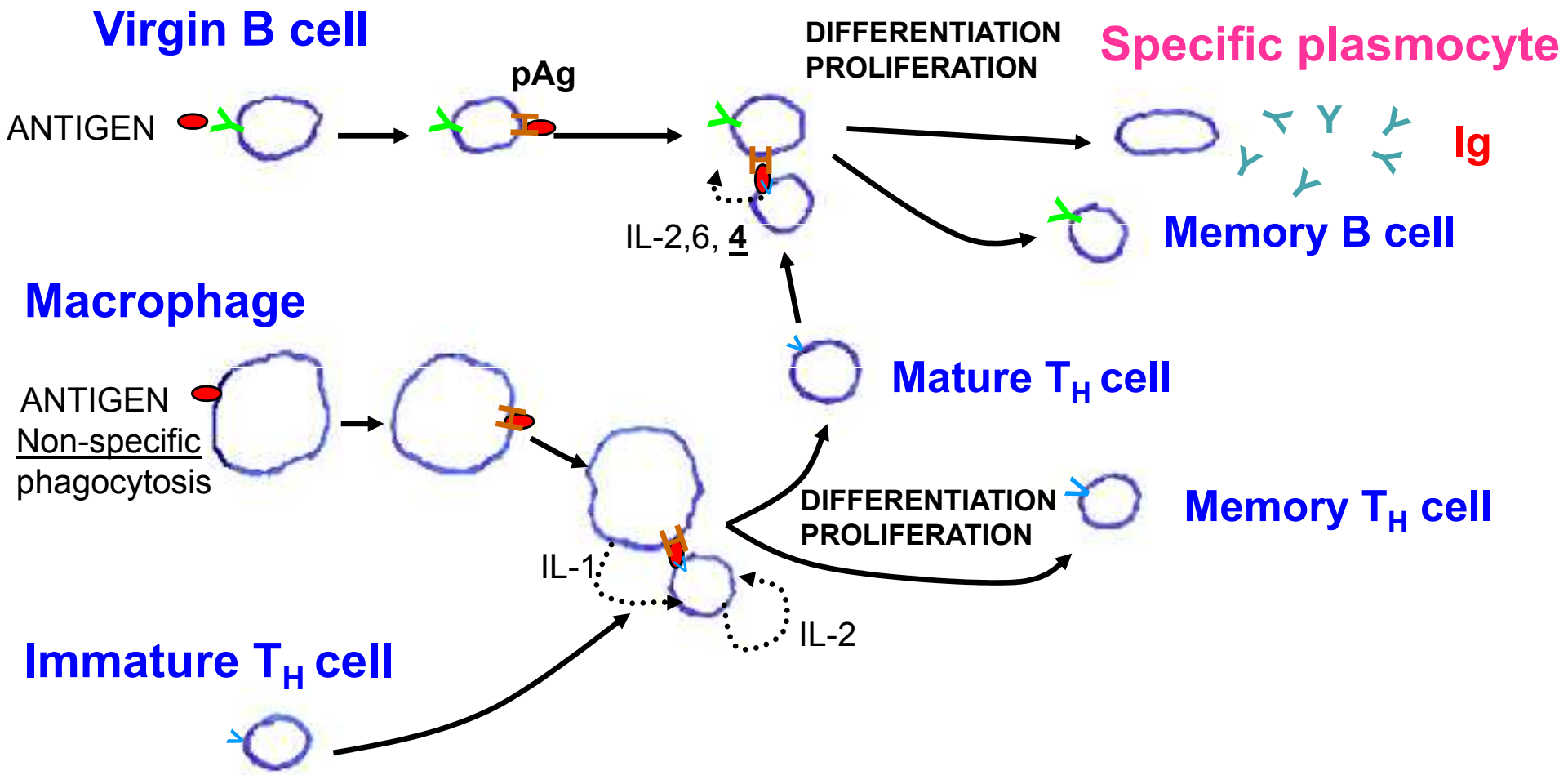
The sources of this diversity are both the **combinatorial association of short gene segments** encoding variable-region genes and the high rate of introduction of **somatic mutations** into the recombined genes.

The κ light-chain gene (on chromosome 2) includes an array of 40 segments V that encode the variable region, 5 segments J that encode the joining region (between the V_L and C_L domains, and a single region that encodes the constant domain C_L. The arrangement of the λ light-chain gene (on chromosome 22) is similar.

The heavy-chain gene (on chromosome 14) includes 51 segments V for the variable regions, 27 segments D (diversity genes), 6 segments J for the joining region, and further groups of segments encoding the heavy-chain constant domains C_H1-4 of distinct immunoglobulin classes.

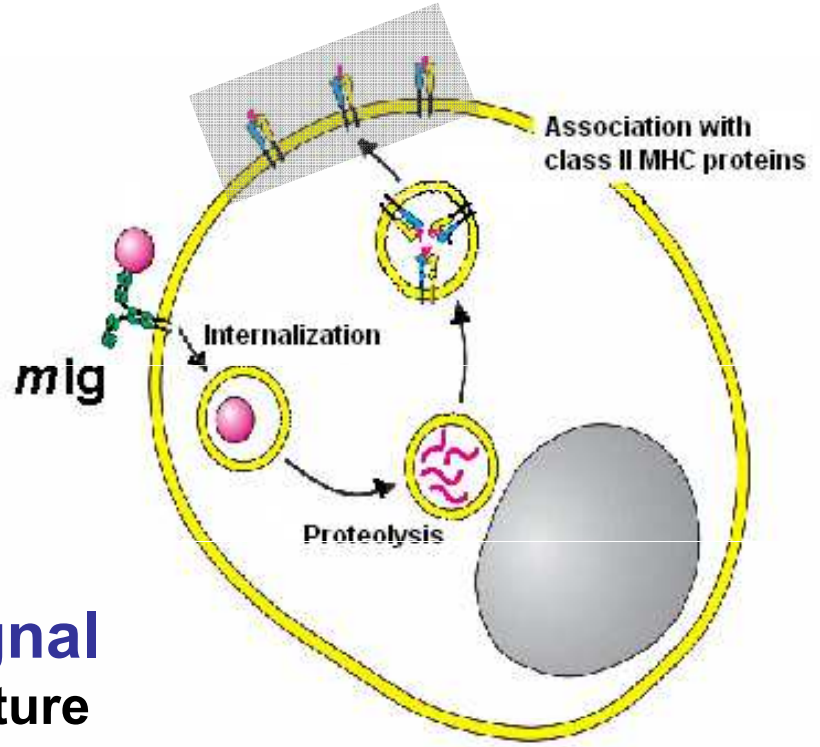
Activation of B lymphocytes – transformation to plasmocytes

The simplified diagram shows the T_H cell-dependent B cell activation by the antigen:



The first specific activation signal evoking the transformation of B cells to plasmocytes is the **binding of specific antigens** (e.g. molecules of soluble foreign proteins on the membrane-bound immunoglobulins (***m*Ig**) on the surfaces of "virgin“ B cells. After internalization by endocytosis, they are digested and peptides with antigenic determinants – **processed antigens** (pAg) – are associated with class II MHC (**m**ajor **h**istocompatibility **c**omplex) proteins, move to the cell surface where they are displayed.

The antigen-presenting B lymphocyte



Continuation of B cells transformation is triggered by **the second activation signal - the binding of the T-cell receptor of a mature helper T cell** with corresponding specifity **to the antigen-presenting B cell**, which results in the **secretion of cytokines**. These cytokines bind to cytokine receptors expressed on the surface of B cells, stimulating differentiation and antibody secretion.

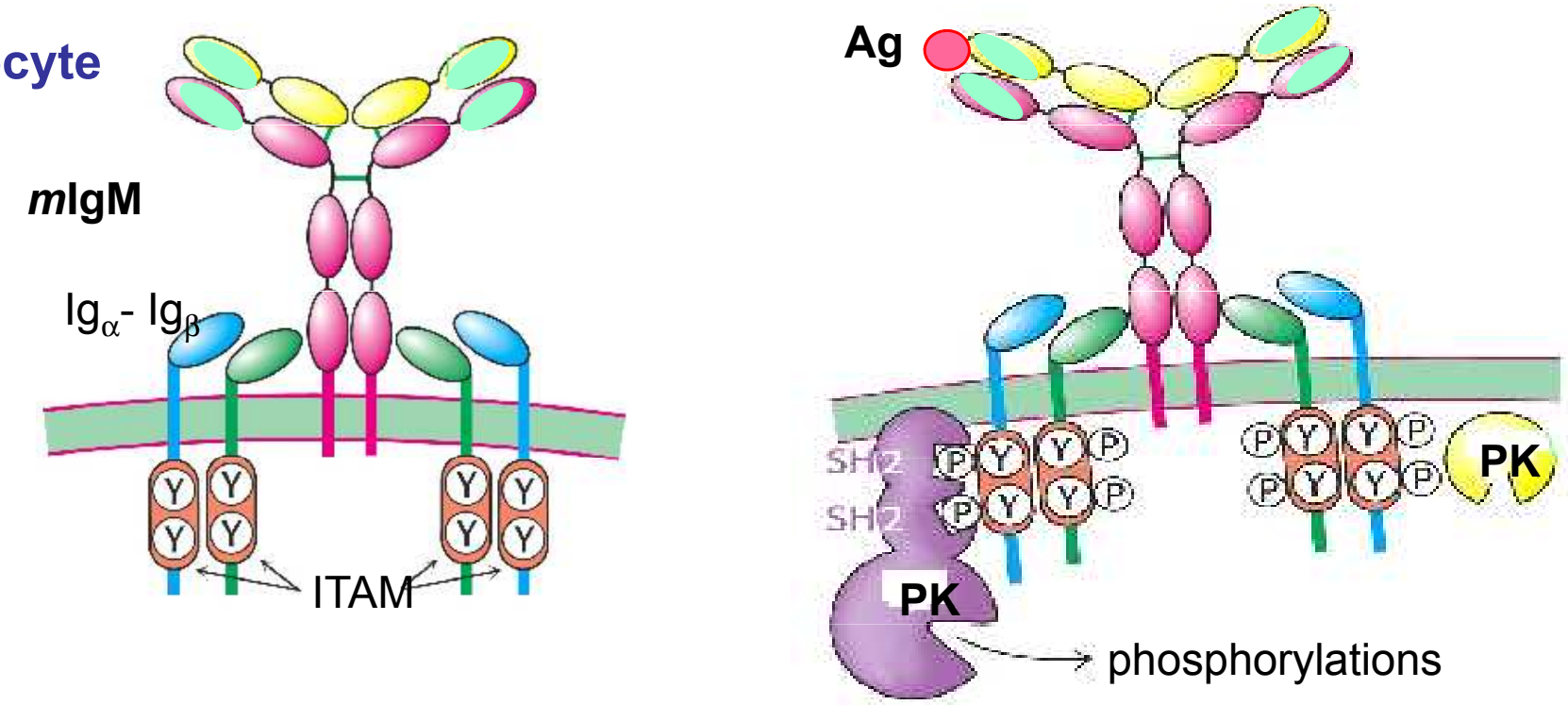
(The immature helper T cell has been transformed to the mature cell by the interaction of its receptor TCR with the same antigenic determinant that was presented in complex with II class MHC protein by a nonspecific-phagocytosing macrophage.)

Some details for thoughtful students:

The first activation signal – the binding of specific antigen to *m*Ig of B cells:

On the surface of each B cell, there are about 10^5 membrane-bound monomeric ***m*IgM** with the same specific binding sites. Those *m*IgMs are associated with dimeric proteins **Ig_α-Ig_β**, the cytoplasmic domains of which includes sequences ITAM (**i**mmunoreceptor **t**yrosine-based **a**ctivation **m**otifs) with tyrosyl residues (**Y**). The **binding of a soluble antigen** to *m*IgM evokes the activation of a tyrosine proteinkinase (PK) and phosphorylation of tyrosyls Y triggers a cascade of following phosphorylations.

B lymphocyte

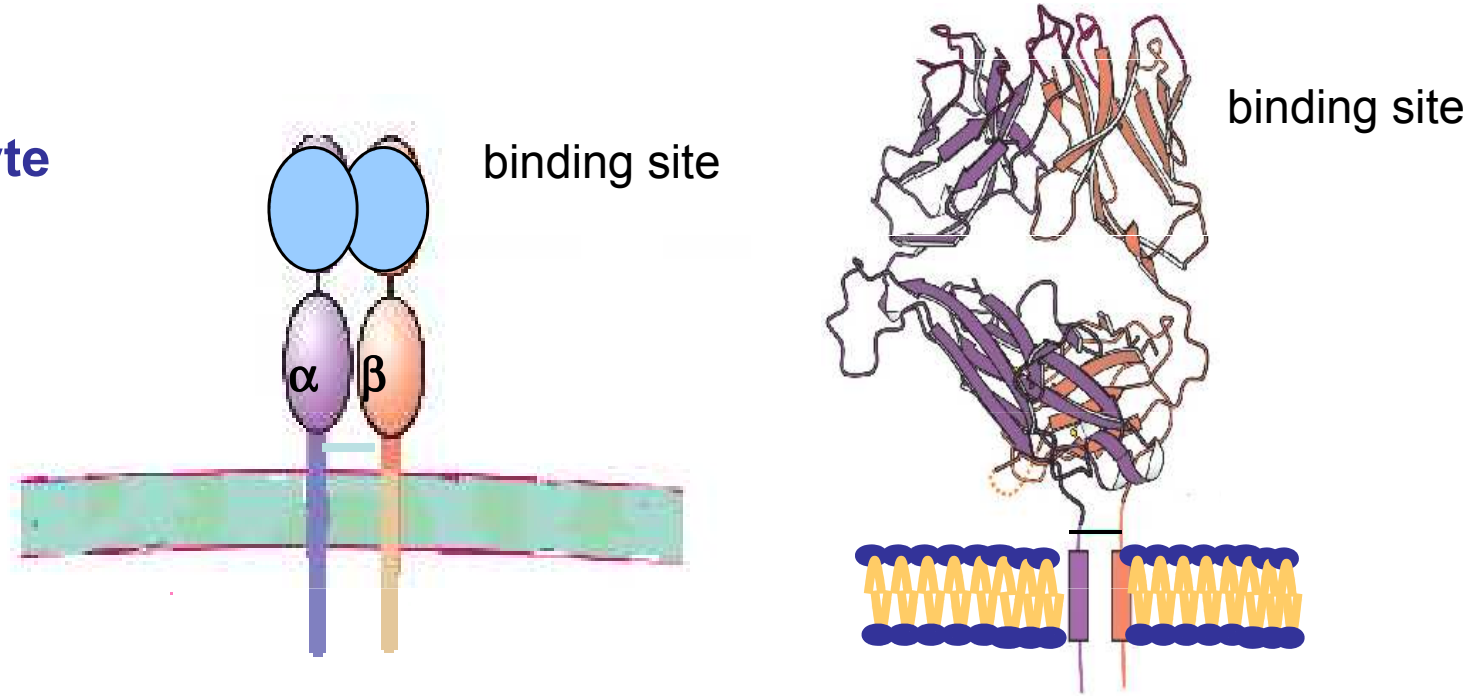


The protein phosphorylations result in the **endocytosis of the Ag-*m*IgM complex**, **processing of the antigen**, and **presentation of the antigenic determinant by means of class II MHC** protein onto the surface of the B cell.

Specific receptors of T cells (TCR)

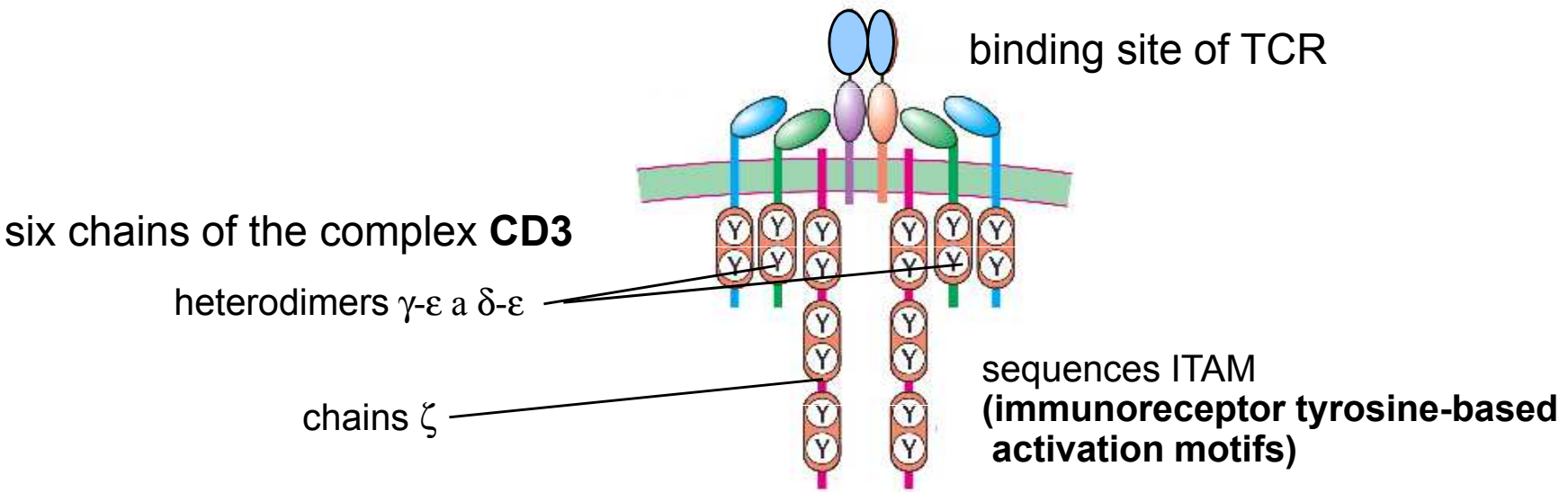
On the surface of each **helper or cytotoxic T cell**, there are numerous membrane receptors TCR. These receptors consist of two chains (α a β) joined by disulfide bridge.
On the outer membrane side, each chain includes two domains (one variable and one constant) that are homologous to the domains of immunoglobulin F_{ab} fragments. Variable domains of both chains form the **monovalent binding site** as in immunoglobulins. All binding sites in the particular clone of T cells exhibit the same specifity.
T cell receptors recognize the corresponding antigenic determinants, but they cannot bind them, unless **these determinants are the components of complexes with MHC proteins on the surface of other antigen-presenting cells**.

T lymphocyte



T cell receptors are **associated with the complex CD3** (**c**luster of **d**ifferentiation **3**) and with either **coreceptor CD4** (in helper T cells) or **coreceptor CD8** (in cytotoxic T cells).

Association of T cell receptors (TCR) with the complex CD3, which consists of six polypeptide chains (two heterodimers γ-ε a δ-ε and two polypeptides ζ). All chains form conspicuous extracellular and cytosolic domains, all cytosolic domains of the complex CD3 include sequences ITAM with tyrosyl residues (Y) that can be phosphorylated.
The chains of heterodimers γ, δ, and ε are nearly the same as the chains Ig_{α} a Ig_{β}, which are associated with membrane immunoglobulins M of B cells.

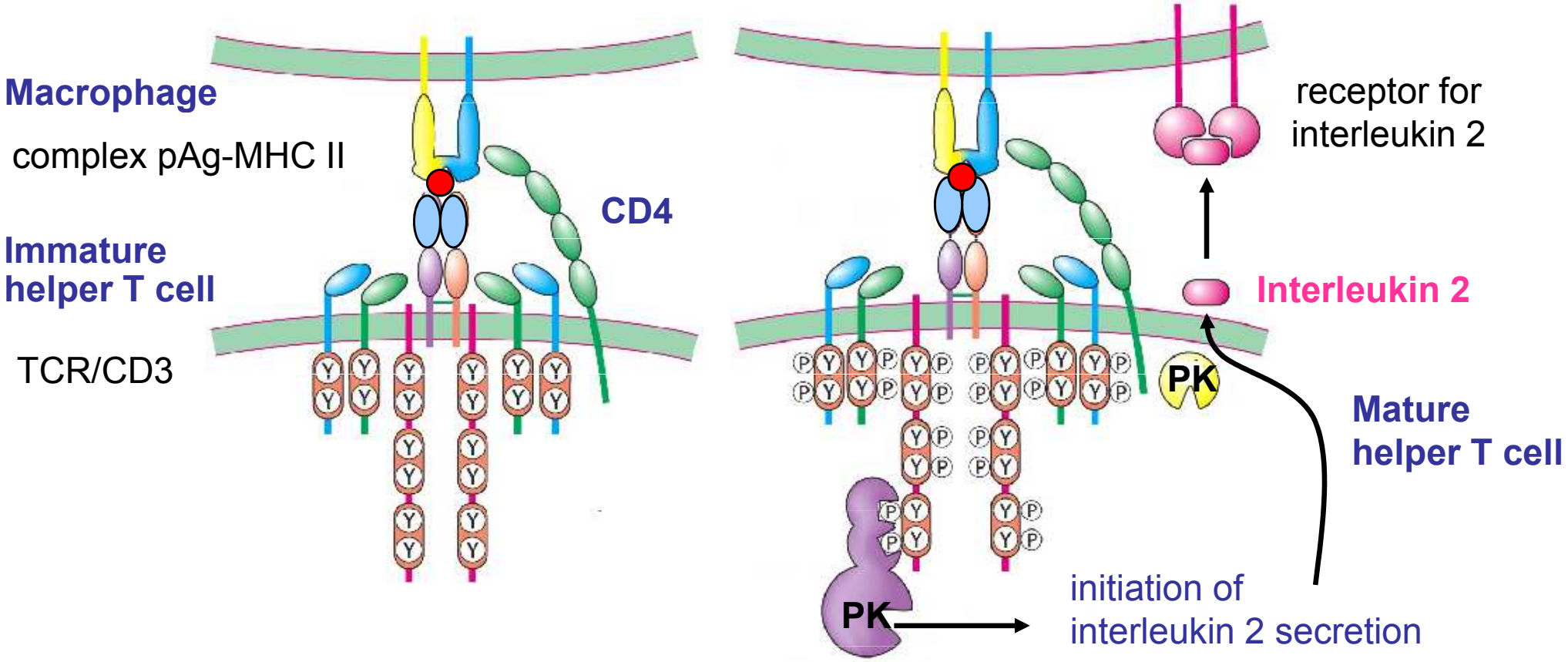


Helper T cell receptors, in cooperation with **coreceptors CD4**, can bind only antigens that are presented as complexes with **class II MHC proteins**, i.e. the antigens presented by macrophages (in triggering of immature helper T cells transformation), B cells (the second signal of B cells activation), and dendritic cells.

Cytotoxic T cell receptors, in cooperation with **coreceptors CD8**, bind determinants presented by **class I MHC** proteins, that are on the surfaces of nearly all cell types.

Maturation of helper T cells **triggered off by the antigen-presenting macrophage**

Antigen being phagocytosed non-specifically by a macrophage is exposed as a processed antigen determinant pAg on the cell surface by means of class II MHC protein. If the determinant is recognized and bound to the receptor of immature helper T cell, the transformation of the T cell into the **mature T cell** is initiated:

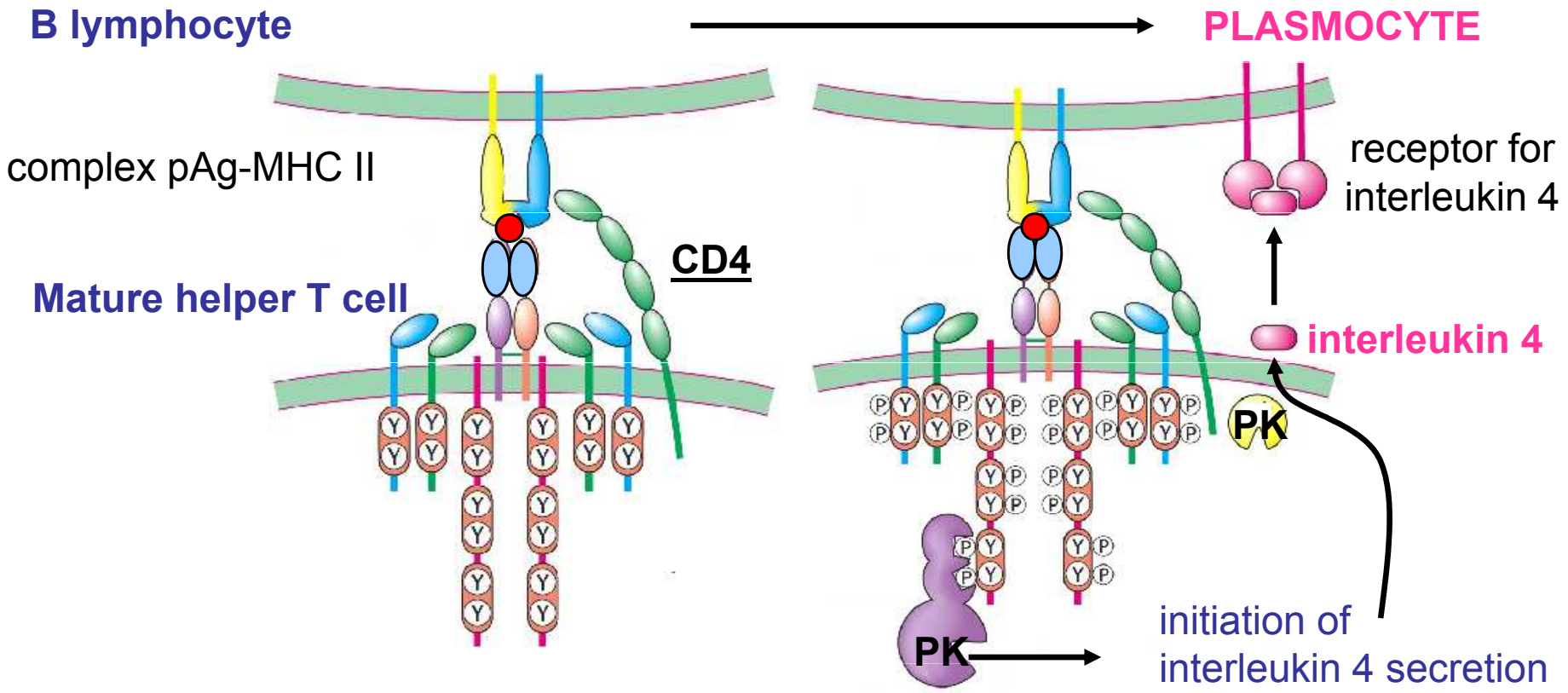


The receptor of mature helper T cell binds to the **antigen-presenting B cell** (in the complex with class II MHC protein - the consequence of the first activation signal). This interaction is the **second activation signal for transformation of the B cell into the plasmocyte**.

The second activation signal

that evokes the transformation of B cells to plasmocytes

is the interaction of mature helper T cell with the antigen determinant presented by the B cell. **Differentiation and proliferation of the B cell** begin and the **B cell is transformed into the plasmocyte** that synthesizes and secretes molecules of immunoglobulins, which are specific for the given antigenic determinant.



Interleukins are proteins secreted from interacting macrophages and immature helper T cells (IL-1 and 2) and from mature helper T cells during their interaction with antigen-presenting B cells (namely IL-4, 2, and 6). They bind to specific types of interleukin receptors and affect significantly the blastic transformation of both helper T cells and B cells.